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(57) Abstract

Novel DNA probe sequences for detection of HIV in a sample in a solution phase sandwich hybridization assay are described. Amplified nucleic acid hybridization assays using the probes are exemplified.

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HIV PROBES FOR USE IN SOLUTION PHASE SANDWICH HYBRIDIZATION ASSAYS

Description

Technical Field

This invention is in the field of nucleic acid hybridization assays. More specifically, it relates to novel nucleic acid probes for detecting Human Immunodeficiency Virus (HIV).

Background Art

The etiological agent of AIDS and ARC has variously been termed LAV, HTLV-III, ARV, and HIV. Hereinafter it will be referred to as HIV. Detection of the RNA or DNA of this virus is possible through a variety of probe sequences and hybridization formats.

PCT WO 88/01302, filed 11 August 1987, discloses thirteen HIV oligonucleotides for use as probes in detecting HIV DNA or RNA. PCT WO 87/07906, filed 22 June 1987, discloses variants of HIV viruses and the use of their DNA to diagnoses AIDS. EP 0 326 395 A2, filed 27 January 1989, discloses an HIV DNA probe spanning nucleotides 2438-2457 for detecting sequences associated with multiple sclerosis.

The advent of the polymerase chain reaction has stimulated a range of assays using probes mainly from regions of the pol and gag genes. Spector et al. (Clin. Chem. 35/8:1581-1587, 1989) and Kellog et al. (Analytical Biochem 189:202-208, 1990) disclose a quantitative assay for HIV proviral DNA using polymerase chain reaction using a primer from the HIV gag gene. Lomell et al.

(Clin. Chem. 35/9:1826-1831) disclose an amplifiable RNA probe complementary to a conserved region of the HIV pol gene mRNA. Coutlee et al. (Anal. Biochem. 181:96-105, 1989) disclose immunodetection of HIV DNA using the polymerase chain reaction with a set of primers complementary to sequences from the HIV pol and gag genes. EP 0 272 098, filed 15 December 1987, discloses PCR amplification and detection of HIV RNA sequences using oligonucleotide probes spanning nucleotides 8538-8547 and 8658-8677. EP 0 229 701, filed 9 January 1987 discloses detection of HIV by amplification of DNA from the HIV gag region. PCT WO 89/10979 discloses a nucleic acid probe assay combining amplification and solution hybridization using capture and reporter probes followed by immobilization on a solid support. A region within the gag p 17 region of HIV was amplified with this technique.

An alternative strategy is termed "reversible target capture." For example, Thompson et al. (Clin. Chem. 35/9:178-1881, 1989) disclose "reversible target capture" of HIV RNA, wherein a commercially available dA-tailed synthetic oligonucleotide provided selective purification of the analyte nucleic acid, and a labeled antisense RNA probe complementary to the HIV pol gene provided signal. Gillespie et al. (Molecular and Cellular Probes 3:73-86, 1989) discloses probes for reversible target capture of HIV RNA, wherein the probes are complementary to nucleotides 2094-4682 of the HIV pol gene.

Kumar et al. disclose a "probe shift" assay for HIV DNA, using DNA sequences complementary to the HIV gag and pol genes. The probe shift assay depends on the hybridization of a labeled oligonucleotide to a PCR-amplified segment in solution. The hemiduplex

thereformed is detected following fractionation on nondenaturing gels.

Keller et al. (Anal. Biochem. 177:27-32, 1989) disclose a microtiter-based sandwich assay to detect HIV DNA spanning the Pst I site of the gag coding region.

Viscidi et al. (J. Clin. Micro. 27:120-125, 1989) disclose a hybridization assay for HIV RNA using a solid phase anti-biotin antibody and an enzyme-labeled monoclonal antibody specific for DNA-RNA hybrids, wherein the probe spanned nearly all of the polymerase gene and the 3' end of the gag gene.

European Patent Application (EPA) 89311862, filed 16 November 1989 discloses a diagnostic kit and method using a solid capture means for detecting nucleic acid, and describes the use of DNA sequences complementary to the HIV gag gene to detect HIV DNA.

Commonly owned U.S. 4,868,105 describes a solution phase nucleic acid sandwich hybridization assay in which analyte nucleic acid is first hybridized in solution to a labeling probe set and to a capturing probe set in a first vessel. The probe-analyte complex is then transferred to a second vessel that contains a solid-phase-immobilized probe that is complementary to a segment of the capturing probes. The segments hybridize to the immobilized probe, thus removing the complex from solution. Having the analyte in the form of an immobilized complex facilitates subsequent separation steps in the assay. Ultimately, single stranded segments of the labeling probe set are hybridized to labeled probes, thus permitting the analyte-containing complex to be detected via a signal generated directly or indirectly from the label.

Commonly owned European Patent Application (EPA) 883096976 discloses a variation in the assay described in U.S. 4,868,105 in which the signal generated

by the labeled probes is amplified. The amplification involves the use of nucleic acid multimers. These multimers are branched polynucleotides that are constructed to have a segment that hybridizes specifically to the analyte nucleic acid or to a nucleic acid (branched or linear) that is bound to the analyte and iterations of a second segment that hybridize specifically to the labeled probe. In the assay employing the multimer, the initial steps of hybridizing the analyte to label or amplifier probe sets and capturing probe sets in a first vessel and transferring the complex to another vessel containing immobilized nucleic acid that will hybridize to a segment of the capturing probes are followed. The multimer is then hybridized to the immobilized complex and the labeled probes in turn hybridized to the second segment iterations on the multimer. Since the multimers provide a large number of sites for label probe attachment, the signal is amplified. Amplifier and capture probe sequences are disclosed for Hepatitis B virus, Neisseria gonorrhoeae, penicillin and tetracycline resistance in N. gonorrhoeae, and Chlamydia trachomatis.

Commonly owned copending application Serial No. 558,897, filed 27 July 1990, describes the preparation of large comb-type branched polynucleotide multimers for use in the above-described solution phase assay. The combs provide greater signal enhancement in the assays than the smaller multimers.

U.S. 5,030,557, filed 24 November 1987, discloses a "helper" oligonucleotide selected to bind to the analyte nucleic acid and impose a different secondary and tertiary structure on the target to facilitate the binding of the probe to the target.

Disclosure of the Invention

One aspect of the invention is a synthetic oligonucleotide useful as an amplifier probe in a sandwich hybridization assay for HIV comprising a first segment having a nucleotide sequence substantially complementary to a segment of HIV nucleic acid; and a second segment having a nucleotide sequence substantially complementary to an oligonucleotide unit of a nucleic acid multimer.

Another aspect of the invention is a synthetic oligonucleotide useful as a capture probe in a sandwich hybridization assay for HIV comprising a first segment having a nucleotide sequence substantially complementary to a segment of HIV nucleic acid; and a second segment having a nucleotide sequence substantially complementary to an oligonucleotide bound to a solid phase.

Another aspect of the invention is a spacer oligonucleotide for use in sandwich hybridizations to detect HIV.

Another aspect of the invention is a solution sandwich hybridization assay for detecting the presence of HIV in a sample, comprising

(a) contacting the sample under hybridizing conditions with an excess of (i) an amplifier probe oligonucleotide comprising a first segment having a nucleotide sequence substantially complementary to a segment of HIV nucleic acid and a second segment having a nucleotide sequence substantially complementary to an oligonucleotide unit of a nucleic acid multimer and (ii) a capture probe oligonucleotide comprising a first segment having a nucleotide sequence that is substantially complementary to a segment of HIV nucleic acid and a second segment that is substantially complementary to an oligonucleotide bound to a solid phase;

(b) contacting the product of step (a) under hybridizing conditions with said oligonucleotide bound to the solid phase;

(c) thereafter separating materials not bound to the solid phase;

(d) contacting the bound product of step (c) under hybridization conditions with the nucleic acid multimer, said multimer comprising at least one oligonucleotide unit that is substantially complementary to the second segment of the amplifier probe polynucleotide and a multiplicity of second oligonucleotide units that are substantially complementary to a labeled oligonucleotide;

(e) removing unbound multimer;

(f) contacting under hybridizing conditions the solid phase complex product of step (e) with the labeled oligonucleotide;

(g) removing unbound labeled oligonucleotide; and

(h) detecting the presence of label in the solid phase complex product of step (g).

Another aspect of the invention is a kit for the detection of HIV in a sample comprising in combination

(i) a set of amplifier probe oligonucleotides wherein the amplifier probe oligonucleotide comprises a first segment having a nucleotide sequence substantially complementary to a segment of HIV nucleic acid and a second segment having a nucleotide sequence substantially complementary to an oligonucleotide unit of a nucleic acid multimer;

(ii) a set of capture probe oligonucleotides wherein the capture probe oligonucleotide comprises a first segment having a nucleotide sequence that is substantially complementary to a segment of HIV nucleic

acid and a second segment that is substantially complementary to an oligonucleotide bound to a solid phase;

(iii) a nucleic acid multimer, said multimer comprising at least one oligonucleotide unit that is substantially complementary to the second segment of the amplifier probe polynucleotide and a multiplicity of second oligonucleotide units that are substantially complementary to a labeled oligonucleotide; and

(iv) a labeled oligonucleotide.

Modes for Carrying out the Invention

Definitions

"Solution phase nucleic acid hybridization assay" intends the assay techniques described and claimed in commonly owned U.S. Patent No. 4,868,105, EPA 883096976, and U.S. Ser. No. 558,897.

A "modified nucleotide" intends a nucleotide monomer that may be stably incorporated into a polynucleotide and which has an additional functional group. Preferably, the modified nucleotide is a 5'-cytidine in which the N^4-position is modified to provide a functional hydroxy group.

An "amplifier multimer" intends a branched polynucleotide that is capable of hybridizing simultaneously directly or indirectly to analyte nucleic acid and to a multiplicity of polynucleotide iterations (i.e, either iterations of another multimer or iterations of a labeled probe). The branching in the multimers is effected through covalent bonds and the multimers are composed of two types of oligonucleotide units that are capable of hybridizing, respectively, to analyte nucleic acid or nucleic acid hybridized to analyte nucleic acid and to a multiplicity of labeled probes. The composition and preparation of such multimers are described in EPA

883096976 and U.S. Serial No. 558,897 filed 27 July 1990, the disclosures of which are incorporated herein by reference.

A "spacer oligonucleotide" is intended as an oligonucleotide which binds to analyte RNA but does not contain any sequences for attachment to a solid phase nor any means for detection by an amplifier probe.

The term "amplifier probe" is intended as a branched or linear polynucleotide that is constructed to have a segment that hybridizes specifically to the analyte nucleic acid and a segment or iterations of a segment that hybridize specifically to an amplifier multimer.

The term "capture probe" is intended as an oligonucleotide having a segment substantially complementary to a nucleotide sequence of the analyte nucleic acid and a segment that is substantially complementary to a nucleotide sequence of a solid-phase-immobilized probe.

"Large" as used herein to describe the comb-type branched polynucleotides of the invention intends a molecule having at least about 15 branch sites and at least about 20 iterations of the labeled probe binding sequence.

"Comb-type" as used herein to describe the structure of the branched polynucleotides of the invention intends a polynucleotide having a linear backbone with a multiplicity of sidechains extending from the backbone.

A "cleavable linker molecule" intends a molecule that may be stably incorporated into a polynucleotide chain and which includes a covalent bond that may be broken or cleaved by chemical treatment or physical treatment such as by irradiation.

All nucleic acid sequences disclosed herein are written in a 5′ to 3′ direction. Nucleotides are designated according to the nucleotide symbols recommended by the IUPAC-IUB Biochemical Nomenclature Commission.

Solution Phase Hybridization Assay

The general protocol for the solution phase sandwich hybridizations is as follows. The analyte nucleic acid is placed in a microtiter well with an excess of two single-stranded nucleic acid probe sets: (1) a set of capture probes, each having a first binding sequence substantially complementary to the analyte and a second binding sequence that is substantially complementary to nucleic acid bound to a solid support, for example, the well surface or a bead, and (2) a set of amplifier probes (branched or linear), each having a first binding sequence that is capable of specific binding to the analyte and a second binding sequence that is capable of specific binding to a segment of the multimer. The resulting product is a three component nucleic acid complex of the two probes hybridized to the analyte by their first binding sequences. The second binding sequences of the probes remain as single-stranded segments as they are not substantially complementary to the analyte. This complex hybridizes to the immobilized probe on the solid surface via the second binding sequence of the capture probe. The resulting product comprises the complex bound to the solid surface via the duplex formed by the oligonucleotide bound to the solid surface and the second binding sequence of the capture probe. Unbound materials are then removed from the surface such as by washing.

The amplification multimer is then added to the bound complex under hybridization conditions to permit the multimer to hybridize to the available second binding

sequence(s) of the amplifier probe of the complex. The resulting complex is then separated from any unbound multimer by washing. The labeled oligonucleotide is then added under conditions which permit it to hybridize to the substantially complementary oligonucleotide units of the multimer. The resulting immobilized labeled nucleic acid complex is then washed to remove unbound labeled oligonucleotide, and read.

The analyte nucleic acids may be from a variety of sources, e.g., biological fluids or solids, and may be prepared for the hybridization analysis by a variety of means, e.g., proteinase K/SDS, chaotropic salts, etc. Also, it may be of advantage to decrease the average size of the analyte nucleic acids by enzymatic, physical or chemical means, e.g., restriction enzymes, sonication, chemical degradation (e.g., metal ions), etc. The fragments may be as small as 0.1 kb, usually being at least about 0.5 kb and may be 1 kb or higher. The analyte sequence is provided in single-stranded form for analysis. Where the sequence is naturally present in single-stranded form, denaturation will not be required. However, where the sequence may be present in double-stranded form, the sequence should be denatured. Denaturation can be carried out by various techniques, such as alkali, generally from about 0.05 to 0.2 M hydroxide, formamide, salts, heat, enzymes, or combinations thereof.

The first binding sequences of the capture probe and amplifier probe that are substantially complementary to the analyte sequence will each be of at least 15 nucleotides, usually at least 25 nucleotides, and not more than about 5 kb, usually not more than about 1 kb, preferably not more than about 100 nucleotides. They will typically be approximately 30 nucleotides. They will normally be chosen to bind to different

sequences of the analyte. The first binding sequences may be selected based on a variety of considerations. Depending upon the nature of the analyte, one may be interested in a consensus sequence, a sequence associated with polymorphisms, a particular phenotype or genotype, a particular strain, or the like.

The number of different amplifier and capture probes used influences the sensitivity of the assay, because the more probe sequences used, the greater the signal provided by the assay system. Furthermore, the use of more probe sequences allows the use of more stringent hybridization conditions, thereby reducing the incidence of false positive results. Thus, the number of probes in a set will be at least one capture probe and at least one amplifier probe, more preferably two capture and two amplifier probes, and most preferably 5-100 capture probes and 5-100 amplifier probes. Oligonucleotide probe sequences for HIV were designed by aligning the DNA sequences of 18 HIV strains from GenBank. Regions of greatest homology within the pol gene were selected as capture probes, while regions of lesser homology were selected as amplifier probes. Very heterogeneous regions were selected as spacer probes. Thus, as more strains of HIV are identified and sequenced, additional probes may be designed or the presently preferred set of probes modified by aligning the sequence of the new strain or isolate with the 18 strains used above and similarly identifying regions of greatest homology and lesser homology.

Spacer oligonucleotides were designed to be added to the hybridization cocktail to protect RNA from possible degradation. Capture probe sequences and label probe sequences were designed so that capture probe sequences were interspersed with label probe sequences,

or so that capture probe sequences were clustered together with respect to label probe sequences.

The presently preferred set of probes and their capture or amplifier regions which hybridize specifically to HIV nucleic acid are listed in Example 2.

The second binding sequences of the capture probe and amplifier probe are selected to be substantially complementary, respectively, to the oligonucleotide bound to the solid surface and to a segment of the multimer and so as to not be encountered by endogenous sequences in the sample/analyte. The second binding sequence may be contiguous to the first binding sequence or be spaced therefrom by an intermediate noncomplementary sequence. The probes may include other noncomplementary sequences if desired. These noncomplementary sequences must not hinder the binding of the binding sequences or cause nonspecific binding to occur.

The capture probe and amplifier probe may be prepared by oligonucleotide synthesis procedures or by cloning, preferably the former.

It will be appreciated that the binding sequences need not have perfect complementarity to provide homoduplexes. In many situations, heteroduplexes will suffice where fewer than about 10% of the bases are mismatches, ignoring loops of five or more nucleotides. Accordingly, as used herein the term "complementary" intends exact complementarity wherein each base within the binding region corresponds exactly, and "substantially complementary" intends 90% or greater homology.

The labeled oligonucleotide will include a sequence substantially complementary to the repeated oligonucleotide units of the multimer. The labeled oligonucleotide will include one or more molecules

("labels"), which directly or indirectly provide a detectable signal. The labels may be bound to individual members of the substantially complementary sequence or may be present as a terminal member or terminal tail having a plurality of labels. Various means for providing labels bound to the oligonucleotide sequences have been reported in the literature. See, for example, Leary et al., Proc. Natl. Acad. Sci. USA (1983) 80:4045; Renz and Kurz, Nucl. Acids Res. (1984) 12:3435; Richardson and Gumport, Nucl. Acids Res. (1983) 11:6167; Smith et al., Nucl. Acids. Res. (1985) 13:2399; Meinkoth and Wahl, Anal. Biochem. (1984) 138:267. The labels may be bound either covalently or non-covalently to the substantially complementary sequence. Labels which may be employed include radionuclides, fluorescers, chemiluminescers, dyes, enzymes, enzyme substrates, enzyme cofactors, enzyme inhibitors, enzyme subunits, metal ions, and the like. Illustrative specific labels include fluorescein, rhodamine, Texas red, phycoerythrin, umbelliferone, luminol, NADPH, α-ß-galactosidase, horse-radish peroxidase, alkaline phosphatase, etc.

The ratio of capture probe and amplifier probe to anticipated moles of analyte will each be at least stoichiometric and preferably in excess. This ratio is preferably at least about 1.5:1, and more preferably at least 2:1. It will normally be in the range of 2:1 to 10,000:1. Concentrations of each of the probes will generally range from about 10^{-5} to 10^{-9} M, with sample nucleic acid concentrations varying from 10^{-21} to 10^{-12} M. The hybridization steps of the assay will generally take from about 10 minutes to 20 hours, frequently being completed in about 1 hour. Hybridization can be carried out at a mildly elevated temperature, generally in the range from about 20°C to 80°C, more usually from about 35°C to 70°C, particularly 65°C.

The hybridization reactions are usually done in an aqueous medium, particularly a buffered aqueous medium, which may include various additives. Additives which may be employed include low concentrations of detergent (0.1 to 1%), salts, e.g., sodium citrate (0.017 to 0.17 M), Ficoll, polyvinylpyrrolidone, carrier nucleic acids, carrier proteins, etc. Nonaqueous solvents may be added to the aqueous medium, such as dimethylformamide, dimethylsulfoxide, alcohols, and formamide. These other solvents are generally present in amounts ranging from 2 to 50%.

The stringency of the hybridization medium may be controlled by temperature, salt concentration, solvent system, and the like. Thus, depending upon the length and nature of the sequence of interest, the stringency will be varied.

Depending upon the nature of the label, various techniques can be employed for detecting the presence of the label. For fluorescers, a large number of different fluorometers are available. For chemiluminescers, luminometers or films are available. With enzymes, a fluorescent, chemiluminescent, or colored product can be provided and determined fluorometrically, luminometrically, spectrophotometrically or visually. The various labels which have been employed in immunoassays and the techniques applicable to immunoassays can be employed with the subject assays.

The following examples further illustrate the invention. These examples are not intended to limit the invention in any manner.

EXAMPLES

Example I

Synthesis of Comb-type Branched Polynucleotide

This example illustrates the synthesis of a comb-type branched polynucleotide having 15 branch sites and sidechain extensions having three labeled probe binding sites. This polynucleotide was designed to be used in a solution phase hybridization as described in EPA 883096976.

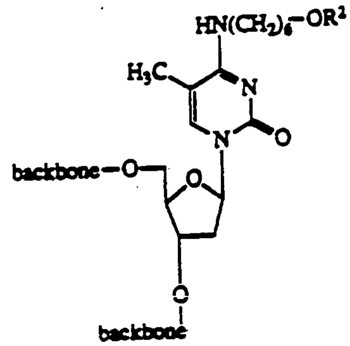
All chemical syntheses of oligonucleotides were performed on an automatic DNA synthesizer (Applied Biosystems, Inc., (ABI) model 380 B). Phosphoramidite chemistry of the beta cyanoethyl type was used including 5'-phosphorylation which employed Phostel™ reagent (ABN). Standard ABI protocols were used except as indicated. Where it is indicated that a multiple of a cycle was used (e.g., 1.2 cycle), the multiple of the standard amount of amidite recommended by ABI was employed in the specified cycle. Appended hereto are the programs for carrying out cycles 1.2 and 6.4 as run on the Applied Biosystems Model 380 B DNA Synthesizer.

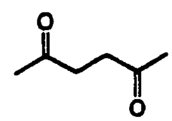
A comb body of the following structure was first prepared:

$$\begin{array}{l} 3'T_{18}(TTX')_{15}GTTTGTGG\text{-}5' \\ \qquad\qquad | \\ \qquad\quad (RGTCAGTp\text{-}5')_{15} \end{array}$$

wherein X' is a branching monomer, and R is a periodate cleavable linker.

The portion of the comb body through the 15 (TTX') repeats is first synthesized using 33.8 mg aminopropyl-derivatized thymidine controlled pore glass (CPG) (2000 Å, 7.4 micromoles thymidine per gram support) with a 1.2 cycle protocol. The branching site nucleotide was of the formula:



where R^2 represents 

For synthesis of the comb body (not including sidechains), the concentration of beta cyanoethylphosphoramidite monomers was 0.1 M for A, C, G and T, 0.15 M for the branching site monomer E, and 0.2 M for Phostel™ reagent. Detritylation was done with 3% trichloroacetic acid in methylene chloride using stepped flowthrough for the duration of the deprotection. At the conclusion the 5′ DMT was replaced with an acetyl group.

Cleavable linker R and six base sidechain extensions of the formula 3′-RGTCAGTp (SEQ ID NO:1) were synthesized at each branching monomer site as follows. The base protecting group removal (R^2 in the formula

above) was performed manually while retaining the CPG support in the same column used for synthesizing the comb body. In the case of R^2 = levulinyl, a solution of 0.5 M hydrazine hydrate in pyridine/glacial acetic acid (1:1 v/v) was introduced and kept in contact with the CPG support for 90 min with renewal of the liquid every 15 min, followed by extensive washing with pyridine/glacial acetic acid (1:1 v/v) and then by acetonitrile. After the deprotection the cleavable linker R and six base sidechain extensions were added using a 6.4 cycle.

In these syntheses the concentration of phosphoramidites was 0.1 M (except 0.2 M R and Phostel™ reagent; R was 2-(4-(4-(2-Dimethoxytrityloxy)ethyl-)phenoxy 2,3-di(benzoyloxy)-butyloxy)phenyl)ethyl-2-cyanoethyl-N,N-diisopropylphosphoramidite).

Detritylation is effected with a solution of 3% trichloroacetic acid in methylene chloride using continuous flowthrough, followed by a rinse solution of toluene/chloromethane (1:1 v/v). Branched polynucleotide chains were removed from the solid supports automatically in the 380B using the cycle "CE NH_3." The ammonium hydroxide solution was collected in 4 ml screw-capped Wheaton vials and heated at 60°C for 12 hr to remove all base-protecting groups. After cooling to room temperature the solvent was removed in a Speed-Vac evaporator and the residue dissolved in 100 µl water.

3' backbone extensions (segment A), sidechain extensions and ligation template/linkers of the following structures were also made using the automatic synthesizer:

3′ Backbone extension	3′-TCCGTATCCTGGGCACAGAGGTGCp-5′ (SEQ ID NO:2)
Sidechain extension	3′-GATGCG(TTCATGCTGTTGGTGTAG)$_3$-5′ (SEQ ID NO:3)
Ligation template for linking 3′ backbone extension	3′-AAAAAAAAAAGCACCTp-5′ (SEQ ID NO:4)
Ligation template for linking sidechain extension	3′-CGCATCACTGAC-5′ (SEQ ID NO:5)

The crude comb body was purified by a standard polyacrylamide gel (7% with 7 M urea and 1X TBE running buffer) method.

The 3′ backbone extension and the sidechain extensions were ligated to the comb body as follows. The comb body (4 pmole/µl), 3′ backbone extension (6.25 pmole/µl), sidechain extension (93.75 pmole/µl) and linking template (5 pmole/µl) were combined in 1 mM ATP/ 5 mM DTT/ 50 mM Tris-HCl, pH 8.0/ 10 mM $MgCl_2$/ 2 mM spermidine, with 0.5 units/µl T4 polynucleotide kinase. The mixture was incubated at 37°C for 2 hr, then heated in a water bath to 95°C, and then cooled to below 35°C for about 1 hr. 2 mM ATP, 10 mM DTT, 14% polyethylene glycol, and 0.21 units/µl T4 ligase were added, and the mixture incubated for 16-24 hr at 23°C. The DNA was precipitated in NaCl/ethanol, resuspended in water, and subjected to a second ligation as follows. The mixture was adjusted to 1 mM ATP, 5 mM DTT, 14% polyethylene glycol, 50 mM Tris-HCl, pH 7.5, 10 mM $MgCl_2$, 2 mM spermidine, 0.5 units/ µl T4 polynucleotide kinase, and 0.21 units/ µl T4 ligase were added, and the mixture incubated at 23°C for 16-24 hr. Ligation products were then purified by polyacrylamide gel electrophoresis.

After ligation and purification, a portion of the product was labeled with ^{32}P and subjected to cleavage at the site of R achieved by oxidation with aqueous $NaIO_4$ for 1 hr. The sample was then analyzed by PAGE to determine the number of sidechain extensions incorporated by quantitating the radioactive label in the bands on the gel. The product was found to have a total of 45 labeled probe binding sites.

Example 2

Sandwich Hybridization Assay for HIV DNA using Multimer

This example illustrates the use of the invention in an HIV DNA assay.

A "15 X 3" amplified solution phase nucleic acid sandwich hybridization assay format was employed in this example. The "15 x 3" designation derives from the fact that the format employs two multimers: (1) an amplifier probe having a first segment (A) that binds to HIV nucleic acid and a second segment (B) that hybridizes to (2) an amplifier multimer having a first segment (B*) that hybridizes to the segment (B) and fifteen iterations of a segment (C), wherein segment C hybridizes to three labeled oligonucleotides.

The amplifier and capture probe HIV-specific segments, and their respective names as used in this assay were as follows.

HIV Amplifier Probes

HIV.104 (SEQ ID NO:5)

TTCCTGGCAAAYYYATKTCTYCTAMTACTGTAT

HIV.105 (SEQ ID NO:6)

CTCCAATTCCYCCTATCATTTTTGGYTTCCATY

HIV.106 (SEQ ID NO:7)

KTATYTGATCRTAYTGTCYYACTTTGATAAAAC

HIV.108 (SEQ ID NO:8)
GTTGACAGGYGTAGGTCCTACYAATAYTGTACC

HIV.110 (SEQ ID NO:9)
YTCAATAGGRCTAATKGGRAAATTTAAAGTRCA

HIV.112 (SEQ ID NO:10)
YTCTGTCAATGGCCATTGYTTRACYYTTGGGCC

HIV.113 (SEQ ID NO:11)
TKTACAWATYTCTRYTAATGCTTTTATTTTYTC

HIV.114 (SEQ ID NO:12)
AAYTYTTGAAATYTTYCCTTCCTTTTCCATHTC

HIV.115 (SEQ ID NO:13)
AAATAYKGGAGTATTRTATGGATTYTCAGGCCC

HIV.116 (SEQ ID NO:14)
TCTCCAYTTRGTRCTGTCYTTTTTCTTTATRGC

HIV.117 (SEQ ID NO:15)
TYTYYTATTAAGYTCYCTGAAATCTACTARTTT

HIV.120 (SEQ ID NO:16)
TKTTYTAAARGGYTCYAAGATTTTTGTCATRCT

HIV.121 (SEQ ID NO:17)
CATGTATTGATADATRAYYATKTCTGGATTTTG

HIV.122 (SEQ ID NO:18)
TATYTCTAARTCAGAYCCTACATACAAATCATC

HIV.123 (SEQ ID NO:19)
TCTYARYTCCTCTATTTTTGYTCTATGCTGYYC

HIV.125 (SEQ ID NO:20)
AAGRAATGGRGGTTCTTTCTGATGYTTYTTRTC

HIV.128 (SEQ ID NO:21)
TRGCTGCYCCATCTACATAGAAVGTTTCTGCWC

HIV.130 (SEQ ID NO:22)
GACAACYTTYTGTCTTCCAYTGTYAGTWASATA

HIV.132 (SEQ ID NO:23)
YGAATCCTGYAAVGCTARRTDAATTGCTTGTAA

HIV.133 (SEQ ID NO:24)
YTGTGARTCTGTYACTATRTTTACTTCTRRTCC

HIV.135 (SEQ ID NO:25)
TATTATTTGAYTRACWAWCTCTGATTCACTYTK

HIV.136 (SEQ ID NO:26)
CAGRTARACYTTTTCCTTTTTTATTARYTGYTC

HIV.137 (SEQ ID NO:27)
TCCTCCAATYCCTTTRTGTGCTGGTACCCATGM

HIV.138 (SEQ ID NO:28)
TCCHBBACTGACTAATYTATCTACTTGTTCATT

HIV.139 (SEQ ID NO:29)
ATCTATTCCATYYAAAAATAGYAYYTTYCTGAT

HIV.141 (SEQ ID NO:30)
GTGGYAGRTTAAARTCAYTAGCCATTGCTYTCC

HIV.142 (SEQ ID NO:31)
CACAGCTRGCTACTATTTCYTTYGCTACYAYRG

HIV.144 (SEQ ID NO:32)
RYTGCCATATYCCKGGRCTACARTCTACTTGTC

HIV.145 (SEQ ID NO:33)
DGATWAYTTTTCCTTCYARATGTGTACAATCTA

HIV.146 (SEQ ID NO:34)
CTATRTAKCCACTRGCYACATGRACTGCTACYA

HIV.147 (SEQ ID NO:35)
CYTGYCCTGTYTCTGCTGGRATDACTTCTGCTT

HIV.149 (SEQ ID NO:36)
TGSKGCCATTGTCTGTATGTAYTRYTKTTACTG

HIV.151 (SEQ ID NO:37)
GAATKCCAAATTCCTGYTTRATHCCHGCCCACC

HIV.152 (SEQ ID NO:38)
ATTCYAYTACYCCTTGACTTTGGGGRTTGTAGG

HIV.153 (SEQ ID NO:39)
GBCCTATRATTTKCTTTAATTCHTTATTCATAG

HIV.154 (SEQ ID NO:40)
CTSTCTTAAGRTGYTCAGCYTGMTCTCTTACYT

HIV.155 (SEQ ID NO:41)
TAAAATTGTGRATRAAYACTGCCATTTGTACWG

HIV.156 (SEQ ID NO:42)

CTGCACTGTAYCCCCCAATCCCCCYTYTTCTTT

HIV.157 (SEQ ID NO:43)
TGTCTGTWGCTATYATRYCTAYTATTCTYTCCC

HIV.158 (SEQ ID NO:44)
TTRTRATTTGYTTTTGTARTTCTYTARTTTGTA

HIV Capture Probes

HIV.103 (SEQ ID NO:45)
CATCTGCTCCTGTRTCTAATAGAGCTTCYTTTA

HIV.111 (SEQ ID NO:46)
ATCCATYCCTGGCTTTAATTTTACTGGTACAGT

HIV.118 (SEQ ID NO:47)
TATTCCTAAYTGRACTTCCCARAARTCYTGAGT

HIV.119 (SEQ ID NO:48)
ACWYTGGAATATYGCYGGTGATCCTTTCCAYCC

HIV.126 (SEQ ID NO:49)
CCATTTRTCAGGRTGGAGTTCATAMCCCATCCA

HIV.127 (SEQ ID NO:50)
CTAYTATGGGKTCYKTYTCTAACTGGTACCAYA

HIV.134 (SEQ ID NO:51)
ATCTGGTTGTGCTTGAATRATYCCYARTGCATA

HIV.143 (SEQ ID NO:52)
CATGCATGGCTTCYCCTTTTAGYTGRCATTTAT

HIV.150 (SEQ ID NO:53)
AACAGGCDGCYTTAACYGYAGYACTGGTGAAAT

HIV.159 (SEQ ID NO:54)
TGTCYCTGTAATAAACCCGAAAATTTTGAATTT

Each amplifier probe contained, in addition to the sequences substantially complementary to the HIV sequences, the following 5' extension complementary to a segment of the amplifier multimer,

AGGCATAGGACCCGTGTCTT (SEQ ID NO:55).

Each capture probe contained, in addition to the sequences substantially complementary to HIV DNA, the following downstream sequence complementary to DNA bound to the solid phase (XT1*),

CTTCTTTGGAGAAAGTGGTG (SEQ ID NO:56).

In addition to the amplifier and capture probes, the following set of HIV spacer oligonucleotides was included in the hybridization mixture.

HIV Spacer Oligonucleotides

HIV.NOX107 (SEQ ID NO:57)
TATAGCTTTHTDTCCRCAGATTTCTAYRR,

HIV.NOX109 (SEQ ID NO:58)
VCCAAKCTGRGTCAACADATTTCKTCCRATTAT,

HIV.NOX124 (SEQ ID NO:59)
TGGTGTGGTAARYCCCCACYTYAAYAGATGYYS,

HIV.NOX129 (SEQ ID NO:60)
TCCTGCTTTTCCYWDTYTAGTYTCYCTRY,

HIV.NOX131 (SEQ ID NO:61)
YTCAGTYTTCTGATTTGTYGTDTBHKTNADRGD,

HIV.NOX140 (SEQ ID NO:62)
AATTRYTGTGATATTTYTCATGDTCHTCTTGRGCCTT,

HIV.NOX148 (SEQ ID NO:63)
GCCATCTKCCTGCTAATTTTARDAKRAARTATGCTGTYT.

Microtiter plates were prepared as follows. White Microlite 1 Removawell strips (polystyrene microtiter plates, 96 wells/plate) were purchased from Dynatech Inc. Each well was filled with 200 µl 1 N HCl and incubated at room temperature for 15-20 min. The plates were then washed 4 times with 1X PBS and the wells aspirated to remove liquid. The wells were then filled with 200 µl 1 N NaOH and incubated at room temperature

for 15-20 min. The plates were again washed 4 times with 1X PBS and the wells aspirated to remove liquid.

Poly(phe-lys) was purchased from Sigma Chemicals, Inc. This polypeptide has a 1:1 molar ratio of phe:lys and an average m.w. of 47,900 gm/mole. It has an average length of 309 amino acids and contains 155 amines/mole. A 1 mg/ml solution of the polypeptide was mixed with 2M NaCl/1X PBS to a final concentration of 0.1 mg/ml (pH 6.0). 200 µL of this solution was added to each well. The plate was wrapped in plastic to prevent drying and incubated at 30°C overnight. The plate was then washed 4 times with 1X PBS and the wells aspirated to remove liquid.

The following procedure was used to couple the oligonucleotide XT1* to the plates. Synthesis of XT1* was described in EPA 883096976. 20 mg disuccinimidyl suberate was dissolved in 300 µl dimethyl formamide (DMF). 26 OD_{260} units of XT1* was added to 100 µl coupling buffer (50 mM sodium phosphate, pH 7.8). The coupling mixture was then added to the DSS-DMF solution and stirred with a magnetic stirrer for 30 min. An NAP-25 column was equilibrated with 10 mM sodium phosphate, pH 6.5. The coupling mixture DSS-DMF solution was added to 2 ml 10 mM sodium phosphate, pH 6.5, at 4°C. The mixture was vortexed to mix and loaded onto the equilibrated NAP-25 column. DSS-activated XT1* DNA was eluted from the column with 3.5 ml 10 mM sodium phosphate, pH 6.5. 5.6 OD_{260} units of eluted DSS-activated XT1* DNA was added to 1500 ml 50 mM sodium phosphate, pH 7.8. 50 µl of this solution was added to each well and the plates were incubated overnight. The plate was then washed 4 times with 1X PBS and the wells aspirated to remove liquid.

Final stripping of plates was accomplished as follows. 200 µL of 0.2N NaOH containing 0.5% (w/v) SDS

was added to each well. The plate was wrapped in plastic and incubated at 65°C for 60 min. The plate was then washed 4 times with 1X PBS and the wells aspirated to remove liquid. The stripped plate was stored with desiccant beads at 2-8°C.

A standard curve of HIV DNA was prepared by diluting cloned HIV DNA in HIV negative human serum and delivering aliquots of dilutions corresponding to a range of 10 to 200 tmoles (1 tmole = 602 molecules or 10^{-21} moles) to wells of microtiter dishes prepared as described above.

Sample preparation consisted of delivering 12.5 µl P-K Buffer (2 mg/ml proteinase K in 10 mM Tris-HCl, pH 8.0/0.15 M NaCl/10 mM EDTA, pH 8.0/1%SDS/40µg/ml sonicated salmon sperm DNA) to each well. Plates were covered and agitated to mix samples, incubated at 65°C to release nucleic acids, and then cooled on the benchtop for 5 min.

A cocktail of the HIV-specific amplifier and capture probes listed above was added to each well (50 fmoles capture probes, 50 fmoles amplifier probes/well). Plates were covered and gently agitated to mix reagents and then incubated at 65°C for 30 min.

Neutralization buffer was then added to each well (0.77 M 3-(N-morpholino)propane sulfonic acid/1.845 M NaCl/0.185 M sodium citrate). Plates were covered and incubated for 12-18 hr at 65°C.

The contents of each well were aspirated to remove all fluid, and the wells washed 2X with washing buffer (0.1% SDS/0.015 M NaCl/ 0.0015 M sodium citrate).

The amplifier multimer was then added to each well (40 µl of 2.5 fmole/µl solution in 50% horse serum/0.06 M NaCl/0.06 M sodium citrate/0.1% SDS mixed 1:1 with 4X SSC/0.1% SDS/0.5% "blocking reagent" (Boehringer Mannheim, catalog No. 1096 176). After

covering plates and agitating to mix the contents in the wells, the plates were incubated for 15 min at 55°C.

After a further 5 min period at room temperature, the wells were washed as described above.

Alkaline phosphatase label probe, disclosed in EP 883096976, was then added to each well (40 μl/well of 2.5 fmoles/μl). After incubation at 55°C for 15 min, and 5 min at room temperature, the wells were washed twice as above and then 3X with 0.015 M NaCl/0.0015 M sodium citrate.

An enzyme-triggered dioxetane (Schaap et al., Tet. Lett. (1987) 28:1159-1162 and EPA Pub. No. 0254051), obtained from Lumigen, Inc., was employed. 20 μl Lumiphos 530 (Lumigen) was added to each well. The wells were tapped lightly so that the reagent would fall to the bottom and gently swirled to distribute the reagent evenly over the bottom. The wells were covered and incubated at 37°C for 40 min.

Plates were then read on a Dynatech ML 1000 luminometer. Output was given as the full integral of the light produced during the reaction.

Results are shown in the Table below. Results for each standard sample are expressed as the difference between the mean of the negative control plus two standard deviations and the mean of the sample minus two standard deviations (delta). If delta is greater than zero, the sample is considered positive.

Results from the standard curve of the HIV probes is shown in Table I. These results indicate the ability of these probe sets to detect 50 tmoles of the HIV DNA standard.

Table I

Analyte HIV tmole/well	Delta
0	---
10	-0.56
20	-0.51
50	0.39
100	1.93
200	5.48

Example 3

Detection of HIV Viral RNA

HIV RNA was detected using essentially the same procedure as above with the following modifications.

A standard curve of HIV RNA was prepared by serially diluting HIV virus stock in normal human serum to a range between 125 to 5000 $TCID_{50}$/ml ($TCID_{50}$ is the 50% tissue culture infectious dose endpoint). A proteinase K solution was prepared by adding 10 mg proteinase K to 5 ml HIV capture diluent (53 mM Tris-HCl, pH 8/ 10.6 mM EDTA/ 1.3% SDS/ 16 µg/ml sonicated salmon sperm DNA/ 5.3 X SSC/ 1 mg/ml proteinase K) made 7% in formamide stored at -20°C. Equimolar mixtures of capture probes, label probes and spacer oligonucleotides were added to the proteinase K solution such that the final concentration of each probe was 1670 fmoles/ml. After addition of 30 µl of the probe/proteinase K solution to each well of microtiter plates prepared as above, 10 µl of appropriate virus dilutions were added to each well. Plates were covered, shaken to mix and then incubated at 65°C for 16 hr.

Plates were removed from the incubator and cooled on the bench top for 10 min. The wells were washed 2X as described in Example 2 above. The 15 X 3

multimer was diluted to 1 fmole/μl in Amp/Label diluent (prepared by mixing 2.22 ml DEPC-treated H_2O (DEPC is diethylpyrocarbonate), 1.35 ml 10% SDS, 240 μl 1 M Tris pH 8.0, 20 μl horse serum, adjusted to 2 mg/ml in proteinase K and heated to 65°C for 2 hr, then added to 240 μl of 0.1 M PMSF and heated at 37°C for 1 hr, after which was added 4 ml DEPC-treated H_2O, 4 ml 10 % SDS and 8 ml 20X SSC). The diluted 15 X 3 multimer was added at 40 μl/well, the plates sealed, shaken, and incubated at 55°C for 30 min.

The plates were then cooled at room temperature for 10 minutes, and washed as described above. Alkaline phosphatase label probe was diluted to 2.5 fmoles/μl in Amp/Label diluent and 40 μl added to each well. Plates were covered, shaken, and incubated at 55°C for 15 min.

Plates were cooled 10 min at room temperature, washed 2X as above and then 3X with 0.15 M NaCl/0.015 M sodium citrate. Substrate was added and luminescence measured as above. Sensitivity of the assay was about 1.25 $TCID_{50}$, as shown in the Table below.

Table II

$TCID_{50}$	delta
0.00	--
1.25	0.11
2.50	2.60
5.00	6.37
10.00	14.10
50.00	90.70

Example 4

Comparison of Clustered vs Interspersed Probe Sets

HIV RNA was detected using essentially the same procedure as in Example 3, except for the following modifications. The RNA standard was prepared by transcription of a 9.0 KB HIV transcript from plasmid pBHBK10S (Chang, P.S., et al., Clin. Biotech. 2:23, 1990) using T7 RNA polymerase. This HIV RNA was quantitated by hybridization with gag and pol probes captured by HAP chromatography. The RNA standard was serially diluted in the proteinase K diluent described above to a range between 2.5 to 100 atomoles per ml, and the equimolar mixtures of capture probes, label probes, and spacer oligonucleotides were added such that the concentration of each probe was 1670 fmoles/ml. Two arrangements of capture and label probes were tested: scattered capture probes, such that capture probes are interspersed with label probes, and clustered capture probes, such that the capture probes are arranged in contiguous clusters with respect to label probes. The clustered probe sets are shown below.

CLUSTERED HIV CAPTURE PROBES

HIV.116 (SEQ ID NO:14)
TCTCCAYTTRGTRCTGTCYTTTTTCTTTATRGC
HIV.117 (SEQ ID NO:15)
TYTYYTATTAAGYTCYCTGAAATCTACTARTTT
HIV.118 (SEQ ID NO:47)
TATTCCTAAYTGRACTTCCCARAARTCYTGAGT
HIV.119 (SEQ ID NO:48)
ACWYTGGAATATYGCYGGTGATCCTTTCCAYCC
HIV.120 (SEQ ID NO:16)
TKTTYTAAARGGYTCYAAGATTTTTGTCATRCT
HIV.155 (SEQ ID NO:41)
TAAAATTGTGRATRAAYACTGCCATTTGTACWG

HIV.156 (SEQ ID NO:42)
CTGCACTGTAYCCCCCAATCCCCCYTYTTCTTT

HIV.157 (SEQ ID NO:43)
TGTCTGTWGCTATYATRYCTAYTATTCTYTCCC

HIV.158 (SEQ ID NO:44)
TTRTRATTTGYTTTTGTARTTCTYTARTTTGTA

HIV.159 (SEQ ID NO:54)
TGTCYCTGTAATAAACCCGAAAATTTTGAATTT

CLUSTERED HIV AMPLIFIER PROBES

HIV.103 (SEQ ID NO:45)
CATCTGCTCCTGTRTCTAATAGAGCTTCYTTTA

HIV.104 (SEQ ID NO:5)
TTCCTGGCAAAYYYATKTCTYCTAMTACTGTAT

HIV.105 (SEQ ID NO:6)
CTCCAATTCCYCCTATCATTTTTGGYTTCCATY

HIV.106 (SEQ ID NO:7)
KTATYTGATCRTAYTGTCYYACTTTGATAAAAC

HIV.108 (SEQ ID NO:8)
GTTGACAGGYGTAGGTCCTACYAATAYTGTACC

HIV.110 (SEQ ID NO:9)
YTCAATAGGRCTAATKGGRAAATTTAAAGTRCA

HIV.111 (SEQ ID NO:46)
ATCCATYCCTGGCTTTAATTTTACTGGTACAGT

HIV.112 (SEQ ID NO:10)
YTCTGTCAATGGCCATTGYTTRACYYTTGGGCC

HIV.113 (SEQ ID NO:11)
TKTACAWATYTCTRYTAATGCTTTTATTTTYTC

HIV.114 (SEQ ID NO:12)
AAYTYTTGAAATYTTYCCTTCCTTTTCCATHTC

HIV.115 (SEQ ID NO:13)
AAATAYKGGAGTATTRTATGGATTYTCAGGCCC

HIV.121 (SEQ ID NO:17)
CATGTATTGATADATRAYYATKTCTGGATTTTG

HIV.122 (SEQ ID NO:18)

TATYTCTAARTCAGAYCCTACATACAAATCATC

HIV.123 (SEQ ID NO:19)

TCTYARYTCCTCTATTTTTGYTCTATGCTGYYC

HIV.125 (SEQ ID NO:20)

AAGRAATGGRGGTTCTTTCTGATGYTTYTTRTC

HIV.126 (SEQ ID NO:49)

CCATTTRTCAGGRTGGAGTTCATAMCCCATCCA

HIV.127 (SEQ ID NO:50)

CTAYTATGGGKTCYKTYTCTAACTGGTACCAYA

HIV.128 (SEQ ID NO:21)

TRGCTGCYCCATCTACATAGAAVGTTTCTGCWC

HIV.130 (SEQ ID NO:22)

GACAACYTTYTGTCTTCCAYTGTYAGTWASATA

HIV.132 (SEQ ID NO:23)

YGAATCCTGYAAVGCTARRTDAATTGCTTGTAA

HIV.133 (SEQ ID NO:24)

YTGTGARTCTGTYACTATRTTTACTTCTRRTCC

HIV.134 (SEQ ID NO:51)

ATCTGGTTGTGCTTGAATRATYCCYARTGCATA

HIV.135 (SEQ ID NO:25)

TATTATTTGAYTRACWAWCTCTGATTCACTYTK

HIV.136 (SEQ ID NO:26)

CAGRTARACYTTTTCCTTTTTTATTARYTGYTC

HIV.137 (SEQ ID NO:27)

TCCTCCAATYCCTTTRTGTGCTGGTACCCATGM

HIV.138 (SEQ ID NO:28)

TCCHBBACTGACTAATYTATCTACTTGTTCATT

HIV.139 (SEQ ID NO:29)

ATCTATTCCATYYAAAAATAGYAYYTTYCTGAT

HIV.141 (SEQ ID NO:30)

GTGGYAGRTTAAARTCAYTAGCCATTGCTYTCC

HIV.142 (SEQ ID NO:31)

CACAGCTRGCTACTATTTCYTTYGCTACYAYRG

HIV.143 (SEQ ID NO:52)
CATGCATGGCTTCYCCTTTTAGYTGRCATTTAT
HIV.144 (SEQ ID NO:32)
RYTGCCATATYCCKGGRCTACARTCTACTTGTC
HIV.145 (SEQ ID NO:33)
DGATWAYTTTTCCTTCYARATGTGTACAATCTA
HIV.146 (SEQ ID NO:34)
CTATRTAKCCACTRGCYACATGRACTGCTACYA
HIV.147 (SEQ ID NO:35)
CYTGYCCTGTYTCTGCTGGRATDACTTCTGCTT
HIV.149 (SEQ ID NO:36)
TGSKGCCATTGTCTGTATGTAYTRYTKTTACTG
HIV.150 (SEQ ID NO:53)
AACAGGCDGCYTTAACYGYAGYACTGGTGAAAT
HIV.151 (SEQ ID NO:37)
GAATKCCAAATTCCTGYTTRATHCCHGCCCACC
HIV.152 (SEQ ID NO:38)
ATTCYAYTACYCCTTGACTTTGGGGRTTGTAGG
HIV.153 (SEQ ID NO:39)
GBCCTATRATTTKCTTTAATTCHTTATTCATAG
HIV.154 (SEQ ID NO:40)
CTSTCTTAAGRTGYTCAGCYTGMTCTCTTACYT

After addition of 30 μl of the analyte/probe/proteinase K solution to each well, 10 μl of normal human serum was added and the assay carried out as described in Example 3. As shown in Table III, the sensitivity of the assay with scattered versus the clustered capture arrangement was similar. Using the clustered capture extenders sensitivity was 50 to 100 tmoles, whereas using the scattered capture extenders, sensitivity was 100 to 500 tmoles.

Table 3

Probe Arrangement	Analyte tmoles	Delta
Clustered	0	---
	25	-0.16
	50	0.36
	100	0.65
	500	4.45
	1000	6.24
Scattered	0	---
	25	-0.24
	50	0.25
	100	-0.11
	500	2.52
	1000	4.79

Modifications of the above-described modes for carrying out the invention that are obvious to those of skill in biochemistry, nucleic acid hybridization assays, and related fields are intended to be within the scope of the following claims.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

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(ii) TITLE OF INVENTION: HIV PROBES FOR USE IN SOLUTION PHASE SANDWICH HYBRIDIZATION ASSAYS

(iii) NUMBER OF SEQUENCES: 63

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(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
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(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

CGTGGAGACA CGGGTCCTAT GCCT 24

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 60 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

GATGTGGTTG TCGTACTTGA TGTGGTTGTC GTACTTGATG TGGTTGTCGT ACTTGCGTAG 60

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 16 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

TCCACGAAAA AAAAAA 16

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 12 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

CAGTCACTAC GC 12

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 33 base pairs
(B) TYPE: nucleic acid

(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

TTCCTGGCAA AYYYATKTCT YCTAMTACTG TAT 33

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 33 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

CTCCAATTCC YCCTATCATT TTTGGYTTCC ATY 33

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 33 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

KTATYTGATC RTAYTGTCYY ACTTTGATAA AAC 33

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 33 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

GTTGACAGGY GTAGGTCCTA CYAATAYTGT ACC 33

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 33 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

YTCAATAGGR CTAATKGGRA AATTTAAAGT RCA 33

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 33 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

YTCTGTCAAT GGCCATTGYT TRACYYTTGG GCC 33

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 33 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

TKTACAWATY TCTRYTAATG CTTTTATTTT YTC 33

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 33 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

AAYTYTTGAA ATYTTYCCTT CCTTTTCCAT HTC 33

(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 33 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

AAATAYKGGA GTATTRTATG GATTYTCAGG CCC 33

(2) INFORMATION FOR SEQ ID NO:14:

(i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 33 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

TCTCCAYTTR GTRCTGTCYT TTTTCTTTAT RGC 33

(2) INFORMATION FOR SEQ ID NO:15:

(i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 33 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

TYTYYTATTA AGYTCYCTGA AATCTACTAR TTT 33

(2) INFORMATION FOR SEQ ID NO:16:

(i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 33 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

TKTTYTAAAR GGYTCYAAGA TTTTTGTCAT RCT 33

(2) INFORMATION FOR SEQ ID NO:17:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 33 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

CATGTATTGA TADATRAYYA TKTCTGGATT TTG 33

(2) INFORMATION FOR SEQ ID NO:18:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 33 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

TATYTCTAAR TCAGAYCCTA CATACAAATC ATC 33

(2) INFORMATION FOR SEQ ID NO:19:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 33 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

TCTYARYTCC TCTATTTTTG YTCTATGCTG YYC 33

(2) INFORMATION FOR SEQ ID NO:20:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 33 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

AAGRAATGGR GGTTCTTTCT GATGYTTYTT RTC 33

(2) INFORMATION FOR SEQ ID NO:21:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 33 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:

TRGCTGCYCC ATCTACATAG AAVGTTTCTG CWC 33

(2) INFORMATION FOR SEQ ID NO:22:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 33 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:

GACAACYTTY TGTCTTCCAY TGTYAGTWAS ATA 33

(2) INFORMATION FOR SEQ ID NO:23:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 33 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:23:

YGAATCCTGY AAVGCTARRT DAATTGCTTG TAA 33

(2) INFORMATION FOR SEQ ID NO:24:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 33 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:24:

YTGTGARTCT GTYACTATRT TTACTTCTRR TCC 33

(2) INFORMATION FOR SEQ ID NO:25:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 33 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:25:

TATTATTTGA YTRACWAWCT CTGATTCACT YTK 33

(2) INFORMATION FOR SEQ ID NO:26:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 33 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:26:

CAGRTARACY TTTTCCTTTT TTATTARYTG YTC 33

(2) INFORMATION FOR SEQ ID NO:27:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 33 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:27:

TCCTCCAATY CCTTTRTGTG CTGGTACCCA TGM 33

(2) INFORMATION FOR SEQ ID NO:28:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 33 base pairs
(B) TYPE: nucleic acid

(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:28:

TCCHBBACTG ACTAATYTAT CTACTTGTTC ATT 33

(2) INFORMATION FOR SEQ ID NO:29:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 33 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:29:

ATCTATTCCA TYYAAAAATA GYAYYTTYCT GAT 33

(2) INFORMATION FOR SEQ ID NO:30:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 33 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:30:

GTGGYAGRTT AAARTCAYTA GCCATTGCTY TCC 33

(2) INFORMATION FOR SEQ ID NO:31:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 33 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:31:

CACAGCTRGC TACTATTTCY TTYGCTACYA YRG 33

(2) INFORMATION FOR SEQ ID NO:32:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 33 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:32:

RYTGCCATAT YCCKGGRCTA CARTCTACTT GTC 33

(2) INFORMATION FOR SEQ ID NO:33:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 33 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:33:

DGATWAYTTT TCCTTCYARA TGTGTACAAT CTA 33

(2) INFORMATION FOR SEQ ID NO:34:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 33 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:34:

CTATRTAKCC ACTRGCYACA TGRACTGCTA CYA 33

(2) INFORMATION FOR SEQ ID NO:35:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 33 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:35:

CYTGYCCTGT YTCTGCTGGR ATDACTTCTG CTT 33

(2) INFORMATION FOR SEQ ID NO:36:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 33 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:36:

TGSKGCCATT GTCTGTATGT AYTRYTKTTA CTG 33

(2) INFORMATION FOR SEQ ID NO:37:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 33 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:37:

GAATKCCAAA TTCCTGYTTR ATHCCHGCCC ACC 33

(2) INFORMATION FOR SEQ ID NO:38:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 33 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:38:

ATTCYAYTAC YCCTTGACTT TGGGGRTTGT AGG 33

(2) INFORMATION FOR SEQ ID NO:39:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 33 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:39:

GBCCTATRAT TTKCTTTAAT TCHTTATTCA TAG 33

(2) INFORMATION FOR SEQ ID NO:40:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 33 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:40:

CTSTCTTAAG RTGYTCAGCY TGMTCTCTTA CYT 33

(2) INFORMATION FOR SEQ ID NO:41:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 33 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:41:

TAAAATTGTG RATRAAYACT GCCATTTGTA CWG 33

(2) INFORMATION FOR SEQ ID NO:42:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 33 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:42:

CTGCACTGTA YCCCCCAATC CCCCYTYTTC TTT 33

(2) INFORMATION FOR SEQ ID NO:43:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 33 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:43:

TGTCTGTWGC TATYATRYCT AYTATTCTYT CCC 33

(2) INFORMATION FOR SEQ ID NO:44:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 33 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:44:

TTRTRATTTG YTTTTGTART TCTYTARTTT GTA 33

(2) INFORMATION FOR SEQ ID NO:45:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 33 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:45:

CATCTGCTCC TGTRTCTAAT AGAGCTTCYT TTA 33

(2) INFORMATION FOR SEQ ID NO:46:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 33 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:46:

ATCCATYCCT GGCTTTAATT TTACTGGTAC AGT 33

(2) INFORMATION FOR SEQ ID NO:47:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 33 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:47:

TATTCCTAAY TGRACTTCCC ARAARTCYTG AGT 33

(2) INFORMATION FOR SEQ ID NO:48:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 33 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:48:

ACWYTGGAAT ATYGCYGGTG ATCCTTTCCA YCC 33

(2) INFORMATION FOR SEQ ID NO:49:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 33 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:49:

CCATTTRTCA GGRTGGAGTT CATAMCCCAT CCA 33

(2) INFORMATION FOR SEQ ID NO:50:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 33 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:50:

CTAYTATGGG KTCYKTYTCT AACTGGTACC AYA 33

(2) INFORMATION FOR SEQ ID NO:51:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 33 base pairs
(B) TYPE: nucleic acid

(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:51:

ATCTGGTTGT GCTTGAATRA TYCCYARTGC ATA 33

(2) INFORMATION FOR SEQ ID NO:52:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 33 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:52:

CATGCATGGC TTCYCCTTTT AGYTGRCATT TAT 33

(2) INFORMATION FOR SEQ ID NO:53:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 33 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:53:

AACAGGCDGC YTTAACYGYA GYACTGGTGA AAT 33

(2) INFORMATION FOR SEQ ID NO:54:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 33 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:54:

TGTCYCTGTA ATAAACCCGA AAATTTTGAA TTT 33

(2) INFORMATION FOR SEQ ID NO:55:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:55:

AGGCATAGGA CCCGTGTCTT 20

(2) INFORMATION FOR SEQ ID NO:56:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:56:

CTTCTTTGGA GAAAGTGGTG 20

(2) INFORMATION FOR SEQ ID NO:57:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 29 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:57:

TATAGCTTTH TDTCCRCAGA TTTCTAYRR 29

(2) INFORMATION FOR SEQ ID NO:58:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 33 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:58:

VCCAAKCTGR GTCAACADAT TTCKTCCRAT TAT 33

(2) INFORMATION FOR SEQ ID NO:59:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 33 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:59:

TGGTGTGGTA ARYCCCCACY TYAAYAGATG YYS 33

(2) INFORMATION FOR SEQ ID NO:60:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 29 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:60:

TCCTGCTTTT CCYWDTYTAG TYTCYCTRY 29

(2) INFORMATION FOR SEQ ID NO:61:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 33 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:61:

YTCAGTYTTC TGATTTGTYG TDTBHKTNAD RGD 33

(2) INFORMATION FOR SEQ ID NO:62:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 37 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:62:

AATTRYTGTG ATATTTYTCA TGDTCHTCTT GRGCCTT 37

(2) INFORMATION FOR SEQ ID NO:63:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 39 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:63:

GCCATCTKCC TGCTAATTTT ARDAKRAART ATGCTGTYT 39

Listings of All

Cycles, Procedures, and Sequences

Used to Synthesize the 15X Comb

Contained on the 3½" floppy disk

for the 380B DNA Synthesizer

5'- GGT GTT TGG TTG TTG TTG TTG TTG TTG TTG TTG TTG

TTG TTG TTG TTG TTG TTT TTT TTT TTT TTT TTT TT -3'

DNA SEQUENCE
VERSION 2.00

SEQUENCE NAME: 15X-2
SEQUENCE LENGTH: 10
DATE: Aug 27, 199
TIME: 14:06
COMMENT:

5'- 77T GAC TGG T -3'

FILE NAME	LAST ACCESS	DATE CREATED	FILE NAME	LAST ACCESS	DATE CREATED
		FILE TYPE:	SYNTHESIS CYCLE		
6.4XSC-5	08 27, 1991	08 27, 1991	6.4XS-5	08 27, 1991	08 27, 1991
1.2XD-6	08 27, 1991	08 27, 1991	1.2X-6	08 27, 1991	08 27, 1991
ssceaf3	01 07, 1990	01 07, 1990	ceaf3	01 07, 1990	01 07, 1990
10ceaf3	01 07, 1990	01 07, 1990	hpaf3	01 07, 1990	01 07, 1990
10hpaf3	01 07, 1990	01 07, 1990	rnaaf3	01 07, 1990	01 07, 1990
10rnaaf3	01 07, 1990	01 07, 1990	sscef3	01 07, 1990	01 07, 1990
cef3	01 07, 1990	01 07, 1990	10cef3	01 07, 1990	01 07, 1990
10hpf3	01 07, 1990	01 07, 1990	rnaf3	01 07, 1990	01 07, 1990
10rnaf3	01 07, 1990	01 07, 1990	ssceaf1	01 07, 1990	01 07, 1990
ceaf1	01 07, 1990	01 07, 1990	10ceaf1	01 07, 1990	01 07, 1990
hpaf1	01 07, 1990	01 07, 1990	10hpaf1	01 07, 1990	01 07, 1990
rnaaf1	01 07, 1990	01 07, 1990	10rnaaf1	01 07, 1990	01 07, 1990
sscef1	01 07, 1990	01 07, 1990	cef1	01 07, 1990	01 07, 1990
10cef1	01 07, 1990	01 07, 1990	10hpf1	01 07, 1990	01 07, 1990
rnaf1	01 07, 1990	01 07, 1990	10rnaf1	01 07, 1990	01 07, 1990
		FILE TYPE:	BOTTLE CHANGE PROCEDURE		
bc 18	07 01, 1986	07 01, 1986	bc 17	07 01, 1986	07 01, 1986
bc 16	07 01, 1986	07 01, 1986	bc 15	07 01, 1986	07 01, 1986
bc 14	07 01, 1986	07 01, 1986	bc 13	07 01, 1986	07 01, 1986
bc 12	07 01, 1986	07 01, 1986	bc 11	07 01, 1986	07 01, 1986
bc 10	07 01, 1986	07 01, 1986	bc 9	07 01, 1986	07 01, 1986
bc 8a	07 01, 1986	07 01, 1986	bc 7	07 01, 1986	07 01, 1986
bc 6	07 01, 1986	07 01, 1986	bc 5	07 01, 1986	07 01, 1986
bc 4	07 01, 1986	07 01, 1986	bc 3	07 01, 1986	07 01, 1986
bc 2	07 01, 1986	07 01, 1986	bc 1	07 01, 1986	07 01, 1986
		FILE TYPE:	END PROCEDURE		
CAP-PRIM	08 27, 1991	08 27, 1991	CE NH3	08 27, 1991	08 27, 1991
deprce	10 08, 1990	10 08, 1990	deprce10	10 08, 1990	10 08, 1990
deprhp	10 08, 1990	10 08, 1990	deprhp10	10 08, 1990	10 08, 1990
deprna	10 08, 1990	10 08, 1990	deprna10	10 08, 1990	10 08, 1990
		FILE TYPE:	BEGIN PROCEDURE		
STD PREP	08 27, 1991	08 27, 1991	phos003	07 01, 1986	07 01, 1986
		FILE TYPE:	SHUT-DOWN PROCEDURE		
clean003	07 01, 1986	07 01, 1986			
		FILE TYPE:	DNA SEQUENCES		
15X-2	08 27, 1991	08 27, 1991	15X-1	08 27, 1991	08 27, 1991

STEP NUMBER	FUNCTION #	NAME	STEP TIME	STEP ACTIVE FOR BASES A	G	C	T	5	6	7	SAFE STEP
1	10	#18 To Waste	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
2	9	#18 To Column	10	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
3	2	Reverse Flush	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
4	1	Block Flush	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
5	5	Advance FC	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
6	28	Phos Prep	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
7	+45	Group 1 On	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
8	90	TET To Column	10	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
9	19	B+TET To Col 1	8	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
10	90	TET To Column	4	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
11	-46	Group 1 Off	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
12	+47	Group 2 On	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
13	90	TET To Column	10	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
14	20	B+TET To Col 2	8	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
15	90	TET To Column	4	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
16	-48	Group 2 Off	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
17	+49	Group 3 On	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
18	90	TET To Column	10	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
19	21	B+TET To Col 3	8	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
20	90	TET To Column	4	Yes	Yees	Yes	Yes	Yes	Yes	Yes	Ye
21	-50	Group 3 Off	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
22	4	Wait	15	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
23	+45	Group 1 On	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
24	90	TET To Column	10	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
25	19	B+TET To Col 1	8	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
26	90	TET To Column	4	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
27	-46	Group 1 Off	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
28	+47	Group 2 On	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
29	90	TET To Column	10	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
30	20	B+TET To Col 2	8	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
31	90	TET To Column	4	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Y s
32	-48	Group 2 Off	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
33	+49	Group 3 On	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
34	90	TET To Column	10	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
35	21	B+TET To Col 3	8	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
36	90	TET To Column	4	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
37	-50	Group 3 Off	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
38	4	Wait	30	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
39	+45	Group 1 On	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
40	90	TET To Column	10	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
41	19	B+TET To Col 1	8	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
42	90	TET To Column	4	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
43	-46	Group 1 Off	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes

(Continued next page.)

STEP NUMBER	FUNCTION #	FUNCTION NAME	STEP TIME	STEP ACTIVE FOR BASES A	G	C	T	5	6	7	SAFE STEP
44	+47	Group 2 On	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
45	90	TET To Column	10	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
46	20	B+TET To Col 2	8	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
47	90	TET To Column	4	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
48	-48	Group 2 Off	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
49	+49	Group 3 On	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
50	90	TET To Column	10	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
51	21	B+TET To Col 3	8	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
52	90	TET To Column	4	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
53	-50	Group 3 Off	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
54	4	Wait	30	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
55	+45	Group 1 On	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
56	90	TET To Column	10	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
57	19	B+TET To Col 1	8	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
58	90	TET To Column	4	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
59	-46	Group 1 Off	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
60	+47	Group 2 On	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
61	90	TET To Column	10	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
62	20	B+TET To Col 2	8	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
63	90	TET To Column	4	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
64	-48	Group 2 Off	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
65	+49	Group 3 On	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
66	90	TET To Column	10	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
67	21	B+TET To Col 3	8	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
68	90	TET To Column	4	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
69	-50	Group 3 Off	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
70	4	Wait	30	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
71	+45	Group 1 On	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
72	90	TET To Column	10	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
73	19	B+TET To Col 1	8	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
74	90	TET To Column	4	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Ye
75	-46	Group 1 Off	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
76	+47	Group 2 On	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
77	90	TET To Column	10	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Ye
78	20	B+TET To Col 2	8	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
79	90	TET To Column	4	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
80	-48	Group 2 Off	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
81	+49	Group 3 On	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
82	90	TET To Column	10	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
83	21	B+TET To Col 3	8	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
84	90	TET To Column	4	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
85	-50	Group 3 Off	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
86	4	Wait	30	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
87	+45	Group 1 On	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
88	90	TET To Column	10	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes

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STEP NUMBER*	FUNCTION #	NAME	STEP TIME	STEP ACTIVE FOR BASES A	G	C	T	5	6	7	SAFE STEP
89	19	B+TET To Col 1	8	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
90	90	TET To Column	4	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
91	-46	Group 1 Off	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
92	+47	Group 2 On	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
93	90	TET To Column	10	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
94	20	B+TET To Col 2	8	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
95	90	TET To Column	4	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
96	-48	Group 2 Off	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
97	+49	Group 3 On	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
98	90	TET To Column	10	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
99	21	B+TET To Col 3	8	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
100	90	TET To Column	4	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
101	-50	Group 3 Off	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
102	4	Wait	30	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
103	+45	Group 1 On	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
104	90	TET To Column	10	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
105	19	B+TET To Col 1	8	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
106	90	TET To Column	4	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
107	-46	Group 1 Off	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
108	+47	Group 2 On	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
109	90	TET To Column	10	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
110	20	B+TET To Col 2	8	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
111	90	TET To Column	4	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
112	-48	Group 2 Off	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
113	+49	Group 3 On	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
114	90	TET To Column	10	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
115	21	B+TET To Col 3	8	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
116	90	TET To Column	4	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
117	-50	Group 3 Off	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Y s
118	4	Wait	30	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
119	+45	Group 1 On	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
120	90	TET To Column	10	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
121	19	B+TET To Col 1	8	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
122	90	TET To Column	4	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
123	-46	Group 1 Off	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
124	+47	Group 2 On	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
125	90	TET To Column	10	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
126	20	B+TET To Col 2	8	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
127	90	TET To Column	4	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Y s
128	-48	Group 2 Off	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Ye
129	+49	Group 3 On	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
130	90	TET To Column	10	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
131	21	B+TET To Col 3	8	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
132	90	TET To Column	4	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
133	-50	Group 3 Off	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes

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STEP NUMBER*	FUNCTION #	NAME	STEP TIME	A	G	C	T	5	6	7	SAFE STEP
134	4	Wait	-30	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
135	10	#18 To Waste	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
136	2	Reverse Flush	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
137	1	Block Flush	4	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
138	81	#15 To Waste	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
139	13	#15 To Column	22	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
140	10	#18 To Waste	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
141	4	Wait	30	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
142	2	Reverse Flush	6	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
143	1	Block Flush	4	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
144	9	#18 To Column	10	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
145	34	Flush to Waste	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
146	9	#18 To Column	10	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
147	2	Reverse Flush	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
148	9	#18 To Column	10	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
149	2	Reverse Flush	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
150	9	#18 To Column	10	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
151	2	Reverse Flush	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
152	1	Block Flush	4	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
153	33	Cycle Entry	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
154	6	Waste-Port	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
155	37	Relay 3 Pulse	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
156	82	#14 To Waste	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
157	30	#17 To Waste	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
158	10	#18 To Waste	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
159	9	#18 To Column	20	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
160	11	#17 To Column	60	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
161	14	#14 To Column	20	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
162	2	Reverse Flush	7	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
163	11	#17 To Column	15	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
164	34	Flush to Waste	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
165	11	#17 To Column	15	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
166	2	Reverse Flush	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
167	14	#14 To Column	20	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
168	34	Flush to Waste	10	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
169	7	Waste-Bottle	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
170	9	#18 To Column	10	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
171	2	Reverse Flush	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
172	9	#18 To Column	10	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
173	2	Reverse Flush	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
174	9	#18 To Column	10	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
175	2	Reverse Flush	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
176	1	Block Flush	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes

STEP NUMBER	FUNCTION #	NAME	STEP TIME	STEP ACTIVE FOR BASES A	G	C	T	5	6	7	SAFE STEP
1	10	#18 To Waste	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
2	9	#18 To Column	10	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
3	2	Reverse Flush	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
4	1	Block Flush	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
5	5	Advance FC	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
6	28	Phos Prep	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
7	+45	Group 1 On	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
8	90	TET To Column	10	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
9	19	B+TET To Col 1	8	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
10	90	TET To Column	4	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
11	-46	Group 1 Off	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
12	+47	Group 2 On	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
13	90	TET To Column	10	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
14	20	B+TET To Col 2	8	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
15	90	TET To Column	4	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
16	-48	Group 2 Off	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
17	+49	Group 3 On	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
18	90	TET To Column	10	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
19	21	B+TET To Col 3	8	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
20	90	TET To Column	4	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
21	-50	Group 3 Off	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
22	4	Wait	15	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
23	+45	Group 1 On	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
24	90	TET To Column	10	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
25	19	B+TET To Col 1	8	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
26	90	TET To Column	4	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
27	-46	Group 1 Off	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
28	+47	Group 2 On	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
29	90	TET To Column	10	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
30	20	B+TET To Col 2	8	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
31	90	TET To Column	4	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
32	-48	Group 2 Off	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Ye
33	+49	Group 3 On	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
34	90	TET To Column	10	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
35	21	B+TET To Col 3	8	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
36	90	TET To Column	4	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
37	-50	Group 3 Off	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Ye
38	4	Wait	30	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
39	+45	Group 1 On	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
40	90	TET To Column	10	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
41	19	B+TET To Col 1	8	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
42	90	TET To Column	4	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
43	-46	Group 1 Off	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes

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STEP NUMBER	FUNCTION #	NAME	STEP TIME	A	G	C	T	5	6	7	SAFE STEP
				STEP ACTIVE FOR BASES							
44	+47	Group 2 On	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
45	90	TET To Column	10	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
46	20	B+TET To Col 2	8	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
47	90	TET To Column	4	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
48	-48	Group 2 Off	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
49	+49	Group 3 On	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
50	90	TET To Column	10	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
51	21	B+TET To Col 3	8	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
52	90	TET To Column	4	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
53	-50	Group 3 Off	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
54	4	Wait	30	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
55	+45	Group 1 On	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
56	90	TET To Column	10	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
57	19	B+TET To Col 1	8	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
58	90	TET To Column	4	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
59	-46	Group 1 Off	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
60	+47	Group 2 On	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
61	90	TET To Column	10	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
62	20	B+TET To Col 2	8	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
63	90	TET To Column	4	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
64	-48	Group 2 Off	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
65	+49	Group 3 On	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
66	90	TET To Column	10	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
67	21	B+TET To Col 3	8	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
68	90	TET To Column	4	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
69	-50	Group 3 Off	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
70	4	Wait	30	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
71	+45	Group 1 On	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
72	90	TET To Column	10	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
73	19	B+TET To Col 1	8	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
74	90	TET To Column	4	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
75	-46	Group 1 Off	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Y s
76	+47	Group 2 On	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
77	90	TET To Column	10	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
78	20	B+TET To Col 2	8	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
79	90	TET To Column	4	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
80	-48	Group 2 Off	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
81	+49	Group 3 On	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
82	90	TET To Column	10	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
83	21	B+TET To Col 3	8	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
84	90	TET To Column	4	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
85	-50	Group 3 Off	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
86	4	Wait	30	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
87	+45	Group 1 On	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Y s
88	90	TET To Column	10	Yes	Yes	Yes	Yes	Yes	Yes	Yes	

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STEP NUMBER	FUNCTION #	NAME	STEP TIME	A	G	C	T	5	6	7	SAFE STEP
89	19	B+TET To Col 1	8	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
90	90	TET To Column	4	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
91	-46	Group 1 Off	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
92	+47	Group 2 On	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Y s
93	90	TET To Column	10	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
94	20	B+TET To Col 2	8	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
95	90	TET To Column	4	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
96	-48	Group 2 Off	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
97	+49	Group 3 On	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
98	90	TET To Column	10	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
99	21	B+TET To Col 3	8	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
100	90	TET To Column	4	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
101	-50	Group 3 Off	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
102	4	Wait	30	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
103	+45	Group 1 On	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
104	90	TET To Column	10	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
105	19	B+TET To Col 1	8	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
106	90	TET To Column	4	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
107	-46	Group 1 Off	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
108	+47	Group 2 On	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
109	90	TET To Column	10	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
110	20	B+TET To Col 2	8	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
111	90	TET To Column	4	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
112	-48	Group 2 Off	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
113	+49	Group 3 On	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
114	90	TET To Column	10	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
115	21	B+TET To Col 3	8	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
116	90	TET To Column	4	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
117	-50	Group 3 Off	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
118	4	Wait	30	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
119	+45	Group 1 On	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
120	90	TET To Column	10	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
121	19	B+TET To Col 1	8	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
122	90	TET To Column	4	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
123	-46	Group 1 Off	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
124	+47	Group 2 On	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
125	90	TET To Column	10	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
126	20	B+TET To Col 2	8	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
127	90	TET To Column	4	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
128	-48	Group 2 Off	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
129	+49	Group 3 On	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
130	90	TET To Column	10	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
131	21	B+TET To Col 3	8	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
132	90	TET To Column	4	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
133	-50	Group 3 Off	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes

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STEP NUMBER*	FUNCTION #	NAME	STEP TIME	A	G	C	T	5	6	7	SAFE STEP
134	4	Wait	30	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
135	16	Cap Prep	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
136	10	#18 To Waste	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
137	2	Reverse Flush	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
138	1	Block Flush	4	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
139	91	Cap To Column	22	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
140	10	#18 To Waste	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
141	4	Wait	30	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
142	2	Reverse Flush	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
143	1	Block Flush	4	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
144	81	#15 To Waste	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
145	13	#15 To Column	22	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
146	10	#18 To Waste	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
147	4	Wait	30	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
148	2	Reverse Flush	6	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
149	1	Block Flush	4	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
150	9	#18 To Column	10	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
151	34	Flush to Waste	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
152	9	#18 To Column	10	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
153	2	Reverse Flush	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
154	9	#18 To Column	10	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
155	2	Reverse Flush	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
156	9	#18 To Column	10	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
157	2	Reverse Flush	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Ye
158	1	Block Flush	4	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
159	33	Cycle Entry	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
160	6	Waste-Port	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
161	37	Relay 3 Pulse	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
162	82	#14 To Waste	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
163	30	#17 To Waste	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
164	10	#18 To Waste	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
165	9	#18 To Column	20	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
166	11	#17 To Column	60	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
167	14	#14 To Column	20	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
168	2	Reverse Flush	7	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
169	11	#17 To Column	15	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
170	34	Flush to Waste	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
171	11	#17 To Column	15	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
172	2	Reverse Flush	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
173	14	#14 To Column	20	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
174	34	Flush to Waste	10	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
175	7	Waste-Bottle	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
176	9	#18 To Column	10	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
177	2	Reverse Flush	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
178	9	#18 To Column	10	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes

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STEP NUMBER	FUNCTION #	NAME	STEP TIME	STEP ACTIVE FOR BASES A	G	C	T	5	6	7	SAFE STEP
179	2	Reverse Flush	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
180	9	#18 To Column	10	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
181	2	Reverse Flush	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
182	1	Block Flush	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes

STEP NUMBER	FUNCTION #	FUNCTION NAME	STEP TIME	STEP ACTIVE FOR BASES A	G	C	T	5	6	7	SAFE STEP
1	10	#18 To Waste	2	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
2	9	#18 To Column	9	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
3	2	Reverse Flush	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
4	1	Block Flush	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
5	5	Advance FC	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
6	28	Phos Prep	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
7	+45	Group 1 On	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
8	90	TET To Column	6	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
9	19	B+TET To Col 1	6	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
10	90	TET To Column	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
11	19	B+TET To Col 1	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
12	90	TET To Column	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
13	19	B+TET To Col 1	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
14	9	#18 To Column	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
15	-46	Group 1 Off	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
16	+47	Group 2 On	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
17	10	#18 To Waste	4	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
18	1	Block Flush	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
19	90	TET To Column	6	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
20	20	B+TET To Col 2	6	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
21	90	TET To Column	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
22	20	B+TET To Col 2	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
23	90	TET To Column	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
24	20	B+TET To Col 2	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
25	9	#18 To Column	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
26	-48	Group 2 Off	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
27	+49	Group 3 On	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
28	10	#18 To Waste	4	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
29	1	Block Flush	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
30	90	TET To Column	6	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
31	21	B+TET To Col 3	6	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
32	90	TET To Column	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
33	21	B+TET To Col 3	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
34	90	TET To Column	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
35	21	B+TET To Col 3	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
36	9	#18 To Column	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
37	-50	Group 3 Off	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
38	4	Wait	20						Yes		Yes
39	2	Reverse Flush	5						Yes		Yes
40	10	#18 To Waste	2						Yes		Yes
41	9	#18 To Column	9						Yes		Yes
42	2	Reverse Flush	5						Yes		Yes
43	10	#18 To Waste	3								

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STEP NUMBER	FUNCTION #	NAME	STEP TIME	A	G	C	T	5	6	7	SAFE STEP
44	1	Block Flush	3						Yes		Yes
45	+45	Group 1 On	1						Yes		Yes
46	90	TET To Column	6						Yes		Yes
47	19	B+TET To Col 1	6						Yes		Yes
48	90	TET To Column	3						Yes		Yes
49	19	B+TET To Col 1	3						Yes		Yes
50	90	TET To Column	3						Yes		Yes
51	19	B+TET To Col 1	3						Yes		Yes
52	9	#18 To Column	1						Yes		Y s
53	-46	Group 1 Off	1						Yes		Yes
54	+47	Group 2 On	1						Yes		Yes
55	10	#18 To Waste	4						Yes		Yes
56	1	Block Flush	3						Yes		Yes
57	90	TET To Column	6						Yes		Yes
58	20	B+TET To Col 2	6						Yes		Yes
59	90	TET To Column	3						Yes		Yes
60	20	B+TET To Col 2	3						Yes		Y s
61	90	TET To Column	3						Yes		Yes
62	20	B+TET To Col 2	3						Yes		Yes
63	9	#18 To Column	1						Yes		Yes
64	-48	Group 2 Off	1						Yes		Ye
65	+49	Group 3 On	1						Yes		Yes
66	10	#18 To Waste	4						Yes		Yes
67	1	Block Flush	3						Yes		Yes
68	90	TET To Column	6						Yes		Yes
69	21	B+TET To Col 3	6						Yes		Yes
70	90	TET To Column	3						Yes		Yes
71	21	B+TET To Col 3	3						Yes		Ye
72	90	TET To Column	3						Yes		Yes
73	21	B+TET To Col 3	3						Yes		Yes
74	9	#18 To Column	1						Yes		Yes
75	-50	Group 3 Off	1						Yes		Yes
76	4	Wait	20						Yes		Yes
77	16	Cap Prep	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
78	2	Reverse Flush	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
79	1	Block Flush	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
80	91	Cap To Column	12	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
81	10	#18 To Waste	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
82	4	Wait	8	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
83	2	Reverse Flush	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
84	81	#15 To Waste	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
85	13	#15 To Column	10	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
86	10	#18 To Waste	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
87	4	Wait	15	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
88	2	Reverse Flush	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes

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STEP NUMBER	FUNCTION #	NAME	STEP TIME	STEP ACTIVE FOR BASES A	G	C	T	5	6	7	SAFE STEP
89	9	#18 To Column	9	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
90	34	Flush to Waste	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
91	9	#18 To Column	9	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
92	2	Reverse Flush	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
93	9	#18 To Column	9	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
94	2	Reverse Flush	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
95	1	Block Flush	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
96	33	Cycle Entry	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
97	9	#18 To Column	9	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
98	2	Reverse Flush	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
99	6	Waste-Port	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
100	30	#17 To Waste	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
101	11	#17 To Column	7	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
102	34	Flush to Waste	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
103	11	#17 To Column	7	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
104	34	Flush to Waste	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
105	11	#17 To Column	7	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
106	34	Flush to Waste	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
107	11	#17 To Column	7	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
108	34	Flush to Waste	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
109	11	#17 To Column	7	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
110	34	Flush to Waste	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
111	11	#17 To Column	7	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
112	34	Flush to Waste	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
113	9	#18 To Column	9	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
114	34	Flush to Waste	7	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
115	7	Waste-Bottle	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
116	9	#18 To Column	9	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
117	2	Reverse Flush	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
118	9	#18 To Column	9	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
119	2	Reverse Flush	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Ye
120	1	Block Flush	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes

STEP NUMBER	FUNCTION #	NAME	STEP TIME	STEP ACTIVE FOR BASES A	6	C	T	5	6	7	SAFE STEP
1	10	#18 To Waste	2	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
2	9	#18 To Column	9	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
3	2	Reverse Flush	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
4	1	Block Flush	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
5	5	Advance FC	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
6	28	Phos Prep	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
7	+45	Group 1 On	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
8	90	TET To Column	6	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
9	19	B+TET To Col 1	6	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
10	90	TET To Column	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
11	19	B+TET To Col 1	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
12	90	TET To Column	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
13	19	B+TET To Col 1	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
14	9	#18 To Column	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
15	-46	Group 1 Off	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
16	+47	Group 2 On	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
17	10	#18 To Waste	4	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
18	1	Block Flush	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
19	90	TET To Column	6	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
20	20	B+TET To Col 2	6	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
21	90	TET To Column	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
22	20	B+TET To Col 2	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
23	90	TET To Column	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
24	20	B+TET To Col 2	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
25	9	#18 To Column	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
26	-48	Group 2 Off	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
27	+49	Group 3 On	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
28	10	#18 To Waste	4	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
29	1	Block Flush	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Y s
30	90	TET To Column	6	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
31	21	B+TET To Col 3	6	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
32	90	TET To Column	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
33	21	B+TET To Col 3	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
34	90	TET To Column	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
35	21	B+TET To Col 3	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
36	9	#18 To Column	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
37	-50	Group 3 Off	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
38	4	Wait	20	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
39	16	Cap Prep	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
40	2	Reverse Flush	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
41	1	Block Flush	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Ye
42	91	Cap To Column	12	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
43	10	#18 To Waste	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes

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STEP NUMBER	FUNCTION #	NAME	STEP TIME	STEP ACTIVE FOR BASES A	G	C	T	5	6	7	SAFE STEP
44	4	Wait	8	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
45	2	Reverse Flush	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
46	91	#15 To Waste	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
47	13	#15 To Column	10	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
48	10	#18 To Waste	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
49	4	Wait	15	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
50	2	Reverse Flush	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
51	9	#18 To Column	9	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
52	34	Flush to Waste	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
53	9	#18 To Column	9	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
54	2	Reverse Flush	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
55	9	#18 To Column	9	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
56	2	Reverse Flush	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
57	1	Block Flush	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
58	33	Cycle Entry	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
59	9	#18 To Column	9	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
60	2	Reverse Flush	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
61	6	Waste-Port	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
62	30	#17 To Waste	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
63	11	#17 To Column	7	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
64	34	Flush to Waste	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
65	11	#17 To Column	7	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
66	34	Flush to Waste	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
67	11	#17 To Column	7	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
68	34	Flush to Waste	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
69	11	#17 To Column	7	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
70	34	Flush to Waste	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
71	11	#17 To Column	7	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
72	34	Flush to Waste	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
73	11	#17 To Column	7	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
74	34	Flush to Waste	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
75	9	#18 To Column	9	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
76	34	Flush to Waste	7	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
77	7	Waste-Bottle	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
78	9	#18 To Column	9	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
79	2	Reverse Flush	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
80	9	#18 To Column	9	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
81	2	Reverse Flush	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
82	1	Block Flush	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes

STEP NUMBER	FUNCTION #	NAME	STEP TIME	STEP ACTIVE FOR BASES A	G	C	T	5	6	7	SAFE STEP
1	10	#18 To Waste	2	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Y s
2	9	#18 To Column	15	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
3	2	Reverse Flush	20	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
4	1	Block Flush	4	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
5	16	Cap Prep	10	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
6	91	Cap To Column	30	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
7	10	#18 To Waste	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
8	1	Block Flush	4	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
9	4	Wait	300	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
10	16	Cap Prep	10	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
11	91	Cap To Column	30	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
12	10	#18 To Waste	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
13	1	Block Flush	4	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
14	4	Wait	300	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
15	2	Reverse Flush	10	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
16	10	#18 To Waste	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
17	9	#18 To Column	15	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
18	2	Reverse Flush	10	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
19	9	#18 To Column	15	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
20	2	Reverse Flush	10	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
21	9	#18 To Column	15	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
22	2	Reverse Flush	10	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
23	9	#18 To Column	15	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
24	2	Reverse Flush	10	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
25	9	#18 To Column	15	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
26	2	Reverse Flush	60	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
27	1	Block Flush	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes

STEP NUMBER	FUNCTION #	NAME	STEP TIME	STEP ACTIVE FOR BASES A	G	C	T	5	6	7	SAFE STEP
1	2	Reverse Flush	60	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
2	27	#10 To Collect	17	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
3	10	#18 To Waste	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
4	1	Block Flush	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
5	4	Wait	660	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
6	27	#10 To Collect	18	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
7	10	#18 To Waste	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
8	1	Block Flush	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
9	4	Wait	660	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
10	27	#10 To Collect	18	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
11	10	#18 To Waste	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
12	1	Block Flush	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
13	4	Wait	660	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
14	27	#10 To Collect	17	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
15	10	#18 To Waste	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
16	1	Block Flush	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
17	4	Wait	660	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
18	8	Flush To CLCT	9	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
19	27	#10 To Collect	14	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
20	8	Flush To CLCT	9	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
21	2	Reverse Flush	60	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
22	1	Block Flush	4	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
23	10	#18 To Waste	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
24	9	#18 To Column	30	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
25	2	Reverse Flush	60	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
26	1	Block Flush	10	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
27	42	#10 Vent	2	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes

Deprotection
Shorter wait compared to ABI

STEP NUMBER	FUNCTION #	NAME	STEP TIME	STEP ACTIVE FOR BASES A	G	C	T	5	6	7	SAFE STEP
1	28	Phos Prep	10	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
2	52	A To Waste	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
3	53	G To Waste	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
4	54	C To Waste	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
5	55	T To Waste	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
6	56	#5 To Waste	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
7	57	#6 To Waste	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
8	58	#7 To Waste	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
9	61	TET To Waste	8	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
10	10	#18 To Waste	10	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
11	16	Cap Prep	10	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
12	59	Cap A To Waste	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Ye
13	60	Cap B To Waste	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
14	81	#15 To Waste	8	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
15	82	#14 To Waste	8	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
16	30	#17 To Waste	10	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
17	10	#18 To Waste	15	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
18	1	Block Flush	15	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes

Claims

1. A synthetic oligonucleotide useful as an amplifier probe in a sandwich hybridization assay for HIV, wherein said oligonucleotide comprises:

a first segment comprising a nucleotide sequence substantially complementary to a segment of HIV nucleic acid; and

a second segment comprising a nucleotide sequence substantially complementary to an oligonucleotide unit of a nucleic acid multimer,

wherein said HIV nucleic acid segment is selected from the group consisting of

CATCTGCTCCTGTRTCTAATAGAGCTTCYTTTA (SEQ ID NO:45),
TTCCTGGCAAAYYYATKTCTYCTAMTACTGTAT (SEQ ID NO:5),
CTCCAATTCCYCCTATCATTTTTGGYTTCCATY (SEQ ID NO:6),
KTATYTGATCRTAYTGTCYYACTTTGATAAAAC (SEQ ID NO:7),
GTTGACAGGYGTAGGTCCTACYAATAYTGTACC (SEQ ID NO:8),
YTCAATAGGRCTAATKGGRAAATTTAAAGTRCA (SEQ ID NO:9),
ATCCATYCCTGGCTTTAATTTTACTGGTACAGT (SEQ ID NO:46),
YTCTGTCAATGGCCATTGYTTRACYYTTGGGCC (SEQ ID NO:10),
TKTACAWATYTCTRYTAATGCTTTTATTTTYTC (SEQ ID NO:11),
AAYTYTTGAAATYTTYCCTTCCTTTTCCATHTC (SEQ ID NO:12),
AAATAYKGGAGTATTRTATGGATTYTCAGGCCC (SEQ ID NO:13),
CATGTATTGATADATRAYYATKTCTGGATTTTG (SEQ ID NO:17),
TATYTCTAARTCAGAYCCTACATACAAATCATC (SEQ ID NO:18),
TCTYARYTCCTCTATTTTTGYTCTATGCTGYYC (SEQ ID NO:19),
AAGRAATGGRGGTTCTTTCTGATGYTTYTTRTC (SEQ ID NO:20),
CCATTTRTCAGGRTGGAGTTCATAMCCCATCCA (SEQ ID NO:49),
CTAYTATGGGKTCYKTYTCTAACTGGTACCAYA (SEQ ID NO:50),
TRGCTGCYCCATCTACATAGAAVGTTTCTGCWC (SEQ ID NO:21),
GACAACYTTYTGTCTTCCAYTGTYAGTWASATA (SEQ ID NO:22),
YGAATCCTGYAAVGCTARRTDAATTGCTTGTAA (SEQ ID NO:23),
YTGTGARTCTGTYACTATRTTTACTTCTRRTCC (SEQ ID NO:24),
ATCTGGTTGTGCTTGAATRATYCCYARTGCATA (SEQ ID NO:51),

TATTATTTGAYTRACWAWCTCTGATTCACTYTK (SEQ ID NO:25),
CAGRTARACYTTTTCCTTTTTTATTARYTGYTC (SEQ ID NO:26),
TCCTCCAATYCCTTTRTGTGCTGGTACCCATGM (SEQ ID NO:27),
TCCHBBACTGACTAATYTATCTACTTGTTCATT (SEQ ID NO:28),
ATCTATTCCATYYAAAAATAGYAYYTTYCTGAT (SEQ ID NO:29),
GTGGYAGRTTAAARTCAYTAGCCATTGCTYTCC (SEQ ID NO:30),
CACAGCTRGCTACTATTTCYTTYGCTACYAYRG (SEQ ID NO:31),
CATGCATGGCTTCYCCTTTTAGYTGRCATTTAT (SEQ ID NO:52),
RYTGCCATATYCCKGGRCTACARTCTACTTGTC (SEQ ID NO:32),
DGATWAYTTTTCCTTCYARATGTGTACAATCTA (SEQ ID NO:33),
CTATRTAKCCACTRGCYACATGRACTGCTACYA (SEQ ID NO:34),
CYTGYCCTGTYTCTGCTGGRATDACTTCTGCTT (SEQ ID NO:35),
TGSKGCCATTGTCTGTATGTAYTRYTKTTACTG (SEQ ID NO:36),
AACAGGCDGCYTTAACYGYAGYACTGGTGAAAT (SEQ ID NO:53),
GAATKCCAAATTCCTGYTTRATHCCHGCCCACC (SEQ ID NO:37),
ATTCYAYTACYCCTTGACTTTGGGGRTTGTAGG (SEQ ID NO:38),
GBCCTATRATTTKCTTTAATTCHTTATTCATAG (SEQ ID NO:39),
CTSTCTTAAGRTGYTCAGCYTGMTCTCTTACYT (SEQ ID NO:40).

2. The synthetic oligonucleotide of claim 1, wherein said second segment comprises
AGGCATAGGACCCGTGTCTT (SEQ ID NO:55).

3. A synthetic oligonucleotide useful as a capture probe in a sandwich hybridization assay for HIV, wherein the synthetic oligonucleotide comprises:

a first segment comprising a nucleotide sequence substantially complementary to a segment of HIV nucleic acid; and

a second segment comprising a nucleotide sequence substantially complementary to an oligonucleotide bound to a solid phase,

wherein said HIV nucleic acid segment is selected from the group consisting of
TCTCCAYTTRGTRCTGTCYTTTTTCTTTATRGC (SEQ ID NO:14),

TYTYYTATTAAGYTCYCTGAAATCTACTARTTT (SEQ ID NO:15),
TATTCCTAAYTGRACTTCCCARAARTCYTGAGT (SEQ ID NO:47),
ACWYTGGAATATYGCYGGTGATCCTTTCCAYCC (SEQ ID NO:48),
TKTTYTAAARGGYTCYAAGATTTTTGTCATRCT (SEQ ID NO:16),
TAAAATTGTGRATRAAYACTGCCATTTGTACWG (SEQ ID NO:41),
CTGCACTGTAYCCCCCAATCCCCCYTYTTCTTT (SEQ ID NO:42),
TGTCTGTWGCTATYATRYCTAYTATTCTYTCCC (SEQ ID NO:43),
TTRTRATTTGYTTTTGTARTTCTYTARTTTGTA (SEQ ID NO:44),
TGTCYCTGTAATAAACCCGAAAATTTTGAATTT (SEQ ID NO:54).

4. The synthetic oligonucleotide of claim 3, wherein said second segment comprises

CTTCTTTGGAGAAAGTGGTG (SEQ ID NO:56).

5. A synthetic oligonucleotide useful as an amplifier probe in a sandwich hybridization assay for HIV, wherein said oligonucleotide comprises:

a first segment comprising a nucleotide sequence substantially complementary to a segment of HIV nucleic acid; and

a second segment comprising a nucleotide sequence substantially complementary to an oligonucleotide unit of a nucleic acid multimer,

wherein said HIV nucleic acid segment is selected from the group consisting of

TTCCTGGCAAAYYYATKTCTYCTAMTACTGTAT (SEQ ID NO:5),
CTCCAATTCCYCCTATCATTTTTGGYTTCCATY (SEQ ID NO:6),
KTATYTGATCRTAYTGTCYYACTTTGATAAAAC (SEQ ID NO:7),
GTTGACAGGYGTAGGTCCTACYAATAYTGTACC (SEQ ID NO:8),
YTCAATAGGRCTAATKGGRAAATTTAAAGTRCA (SEQ ID NO:9),
YTCTGTCAATGGCCATTGYTTRACYYTTGGGCC (SEQ ID NO:10),
TKTACAWATYTCTRYTAATGCTTTTATTTTYTC (SEQ ID NO:11),
AAYTYTTGAAATYTTYCCTTCCTTTTCCATHTC (SEQ ID NO:12),
AAATAYKGGAGTATTRTATGGATTYTCAGGCCC (SEQ ID NO:13),

TCTCCAYTTRGTRCTGTCYTTTTTCTTTATRGC (SEQ ID NO:14),
TYTYYTATTAAGYTCYCTGAAATCTACTARTTT (SEQ ID NO:15),
TKTTYTAAARGGYTCYAAGATTTTTGTCATRCT (SEQ ID NO:16),
CATGTATTGATADATRAYYATKTCTGGATTTTG (SEQ ID NO:17),
TATYTCTAARTCAGAYCCTACATACAAATCATC (SEQ ID NO:18),
TCTYARYTCCTCTATTTTTGYTCTATGCTGYYC (SEQ ID NO:19),
AAGRAATGGRGGTTCTTTCTGATGYTTYTTRTC (SEQ ID NO:20),
TRGCTGCYCCATCTACATAGAAVGTTTCTGCWC (SEQ ID NO:21),
GACAACYTTYTGTCTTCCAYTGTYAGTWASATA (SEQ ID NO:22),
YGAATCCTGYAAVGCTARRTDAATTGCTTGTAA (SEQ ID NO:23),
YTGTGARTCTGTYACTATRTTTACTTCTRRTCC (SEQ ID NO:24),
TATTATTTGAYTRACWAWCTCTGATTCACTYTK (SEQ ID NO:25),
CAGRTARACYTTTTCCTTTTTTATTARYTGYTC (SEQ ID NO:26),
TCCTCCAATYCCTTTRTGTGCTGGTACCCATGM (SEQ ID NO:27),
TCCHBBACTGACTAATYTATCTACTTGTTCATT (SEQ ID NO:28),
ATCTATTCCATYYAAAAATAGYAYYTTYCTGAT (SEQ ID NO:29),
GTGGYAGRTTAAARTCAYTAGCCATTGCTYTCC (SEQ ID NO:30),
CACAGCTRGCTACTATTTCYTTYGCTACYAYRG (SEQ ID NO:31),
RYTGCCATATYCCKGGRCTACARTCTACTTGTC (SEQ ID NO:32),
DGATWAYTTTTCCTTCYARATGTGTACAATCTA (SEQ ID NO:33),
CTATRTAKCCACTRGCYACATGRACTGCTACYA (SEQ ID NO:34),
CYTGYCCTGTYTCTGCTGGRATDACTTCTGCTT (SEQ ID NO:35),
TGSKGCCATTGTCTGTATGTAYTRYTKTTACTG (SEQ ID NO:36),
GAATKCCAAATTCCTGYTTRATHCCHGCCCACC (SEQ ID NO:37),
ATTCYAYTACYCCTTGACTTTGGGGRTTGTAGG (SEQ ID NO:38),
GBCCTATRATTTKCTTTAATTCHTTATTCATAG (SEQ ID NO:39),
CTSTCTTAAGRTGYTCAGCYTGMTCTCTTACYT (SEQ ID NO:40),
TAAAATTGTGRATRAAYACTGCCATTTGTACWG (SEQ ID NO:41),
CTGCACTGTAYCCCCCAATCCCCCYTYTTCTTT (SEQ ID NO:42),
TGTCTGTWGCTATYATRYCTAYTATTCTYTCCC (SEQ ID NO:43),
TTRTRATTTGYTTTTGTARTTCTYTARTTTGTA (SEQ ID NO:44).

6. The synthetic oligonucleotide of claim 5, wherein said second segment comprises

AGGCATAGGACCCGTGTCTT (SEQ ID NO:55).

7. A synthetic oligonucleotide useful as a capture probe in a sandwich hybridization assay for HIV, wherein the synthetic oligonucleotide comprises:

a first segment comprising a nucleotide sequence substantially complementary to a segment of HIV nucleic acid; and

a second segment comprising a nucleotide sequence substantially complementary to an oligonucleotide bound to a solid phase,

wherein said HIV nucleic acid segment is selected from the group consisting of

CATCTGCTCCTGTRTCTAATAGAGCTTCYTTTA (SEQ ID NO:45),
ATCCATYCCTGGCTTTAATTTTACTGGTACAGT (SEQ ID NO:46),
TATTCCTAAYTGRACTTCCCARAARTCYTGAGT (SEQ ID NO:47),
ACWYTGGAATATYGCYGGTGATCCTTTCCAYCC (SEQ ID NO:48),
CCATTTRTCAGGRTGGAGTTCATAMCCCATCCA (SEQ ID NO:49),
CTAYTATGGGKTCYKTYTCTAACTGGTACCAYA (SEQ ID NO:50),
ATCTGGTTGTGCTTGAATRATYCCYARTGCATA (SEQ ID NO:51),
CATGCATGGCTTCYCCTTTTAGYTGRCATTTAT (SEQ ID NO:52),
AACAGGCDGCYTTAACYGYAGYACTGGTGAAAT (SEQ ID NO:53),
TGTCYCTGTAATAAACCCGAAAATTTTGAATTT (SEQ ID NO:54).

8. The synthetic oligonucleotide of claim 7, wherein said second segment comprises

CTTCTTTGGAGAAAGTGGTG (SEQ ID NO:56).

9. A synthetic oligonucleotide useful as a spacer oligonucleotide in a sandwich hybridization assay for HIV, wherein the synthetic oligonucleotide comprises a segment substantially complementary to a segment of HIV nucleic acid, wherein said HIV nucleic acid segment is selected from the group consisting of
TATAGCTTTHTDTCCRCAGATTTCTAYRR (SEQ ID NO:57),
VCCAAKCTGRGTCAACADATTTCKTCCRATTAT (SEQ ID NO:58),

TGGTGTGGTAARYCCCCACYTYAAYAGATGYYS (SEQ ID NO:59),
TCCTGCTTTTCCYWDTYTAGTYTCYCTRY (SEQ ID NO:60),
YTCAGTYTTCTGATTTGTYGTDTBHKTNADRGD (SEQ ID NO:61),
AATTRYTGTGATATTTYTCATGDTCHTCTTGRGCCTT (SEQ ID NO:62),
GCCATCTKCCTGCTAATTTTARDAKRAARTATGCTGTYT (SEQ ID NO:63).

10. A set of synthetic oligonucleotides useful as amplifier probes in a sandwich hybridization assay for HIV, comprising two oligonucleotides, wherein each member of the set comprises

a first segment comprising a nucleotide sequence substantially complementary to a segment of HIV nucleic acid; and

a second segment comprising a nucleotide sequence substantially complementary to an oligonucleotide unit of a nucleic acid multimer,

wherein said HIV nucleic acid segments are

CATCTGCTCCTGTRTCTAATAGAGCTTCYTTTA (SEQ ID NO:45),
TTCCTGGCAAAYYYATKTCTYCTAMTACTGTAT (SEQ ID NO:5),
CTCCAATTCCYCCTATCATTTTTGGYTTCCATY (SEQ ID NO:6),
KTATYTGATCRTAYTGTCYYACTTTGATAAAAC (SEQ ID NO:7),
GTTGACAGGYGTAGGTCCTACYAATAYTGTACC (SEQ ID NO:8),
YTCAATAGGRCTAATKGGRAAATTTAAAGTRCA (SEQ ID NO:9),
ATCCATYCCTGGCTTTAATTTTACTGGTACAGT (SEQ ID NO:46),
YTCTGTCAATGGCCATTGYTTRACYYTTGGGCC (SEQ ID NO:10),
TKTACAWATYTCTRYTAATGCTTTTATTTTYTC (SEQ ID NO:11),
AAYTYTTGAAATYTTYCCTTCCTTTTCCATHTC (SEQ ID NO:12),
AAATAYKGGAGTATTRTATGGATTYTCAGGCCC (SEQ ID NO:13),
CATGTATTGATADATRAYYATKTCTGGATTTTG (SEQ ID NO:17),
TATYTCTAARTCAGAYCCTACATACAAATCATC (SEQ ID NO:18),
TCTYARYTCCTCTATTTTTGYTCTATGCTGYYC (SEQ ID NO:19),
AAGRAATGGRGGTTCTTTCTGATGYTTYTTRTC (SEQ ID NO:20),
CCATTTRTCAGGRTGGAGTTCATAMCCCATCCA (SEQ ID NO:49),
CTAYTATGGGKTCYKTYTCTAACTGGTACCAYA (SEQ ID NO:50),
TRGCTGCYCCATCTACATAGAAVGTTTCTGCWC (SEQ ID NO:21),

GACAACYTTYTGTCTTCCAYTGTYAGTWASATA (SEQ ID NO:22),
YGAATCCTGYAAVGCTARRTDAATTGCTTGTAA (SEQ ID NO:23),
YTGTGARTCTGTYACTATRTTTACTTCTRRTCC (SEQ ID NO:24),
ATCTGGTTGTGCTTGAATRATYCCYARTGCATA (SEQ ID NO:51),
TATTATTTGAYTRACWAWCTCTGATTCACTYTK (SEQ ID NO:25),
CAGRTARACYTTTTCCTTTTTTATTARYTGYTC (SEQ ID NO:26),
TCCTCCAATYCCTTTRTGTGCTGGTACCCATGM (SEQ ID NO:27),
TCCHBBACTGACTAATYTATCTACTTGTTCATT (SEQ ID NO:28),
ATCTATTCCATYYAAAAATAGYAYYTTYCTGAT (SEQ ID NO:29),
GTGGYAGRTTAAARTCAYTAGCCATTGCTYTCC (SEQ ID NO:30),
CACAGCTRGCTACTATTTCYTTYGCTACYAYRG (SEQ ID NO:31),
CATGCATGGCTTCYCCTTTTAGYTGRCATTTAT (SEQ ID NO:52),
RYTGCCATATYCCKGGRCTACARTCTACTTGTC (SEQ ID NO:32),
DGATWAYTTTTCCTTCYARATGTGTACAATCTA (SEQ ID NO:33),
CTATRTAKCCACTRGCYACATGRACTGCTACYA (SEQ ID NO:34),
CYTGYCCTGTYTCTGCTGGRATDACTTCTGCTT (SEQ ID NO:35),
TGSKGCCATTGTCTGTATGTAYTRYTKTTACTG (SEQ ID NO:36),
AACAGGCDGCYTTAACYGYAGYACTGGTGAAAT (SEQ ID NO:53),
GAATKCCAAATTCCTGYTTRATHCCHGCCCACC (SEQ ID NO:37),
ATTCYAYTACYCCTTGACTTTGGGGRTTGTAGG (SEQ ID NO:38),
GBCCTATRATTTKCTTTAATTCHTTATTCATAG (SEQ ID NO:39),
CTSTCTTAAGRTGYTCAGCYTGMTCTCTTACYT (SEQ ID NO:40).

11. The set of synthetic oligonucleotides of claim 10, wherein said second segment comprises AGGCATAGGACCCGTGTCTT (SEQ ID NO:55).

12. A set of synthetic oligonucleotides useful as capture probes in a sandwich hybridization assay for HIV, comprising two oligonucleotides, wherein each member of the set comprises

a first segment comprising a nucleotide sequence substantially complementary to a segment of HIV nucleic acid; and

a second segment comprising a nucleotide sequence substantially complementary to an oligonucleotide bound to a solid phase,

wherein said HIV nucleic acid segments are

TCTCCAYTTRGTRCTGTCYTTTTTCTTTATRGC (SEQ ID NO:14),
TYTYYTATTAAGYTCYCTGAAATCTACTARTTT (SEQ ID NO:15),
TATTCCTAAYTGRACTTCCCARAARTCYTGAGT (SEQ ID NO:47),
ACWYTGGAATATYGCYGGTGATCCTTTCCAYCC (SEQ ID NO:48),
TKTTYTAAARGGYTCYAAGATTTTTGTCATRCT (SEQ ID NO:16),
TAAAATTGTGRATRAAYACTGCCATTTGTACWG (SEQ ID NO:41),
CTGCACTGTAYCCCCCAATCCCCCYTYTTCTTT (SEQ ID NO:42),
TGTCTGTWGCTATYATRYCTAYTATTCTYTCCC (SEQ ID NO:43),
TTRTRATTTGYTTTTGTARTTCTYTARTTTGTA (SEQ ID NO:44),
TGTCYCTGTAATAAACCCGAAAATTTTGAATTT (SEQ ID NO:54).

13. The set of synthetic oligonucleotides of claim 12, wherein said second segment comprises

CTTCTTTGGAGAAAGTGGTG (SEQ ID NO:56).

14. A set of synthetic oligonucleotides useful as amplifier probes in a sandwich hybridization assay for HIV, comprising two oligonucleotides, wherein each member of the set comprises

a first segment comprising a nucleotide sequence substantially complementary to a segment of HIV nucleic acid; and

a second segment comprising a nucleotide sequence substantially complementary to an oligonucleotide unit of a nucleic acid multimer,

wherein said HIV nucleic acid segments are

TTCCTGGCAAAYYYATKTCTYCTAMTACTGTAT (SEQ ID NO:5),
CTCCAATTCCYCCTATCATTTTTGGYTTCCATY (SEQ ID NO:6),
KTATYTGATCRTAYTGTCYYACTTTGATAAAAC (SEQ ID NO:7),
GTTGACAGGYGTAGGTCCTACYAATAYTGTACC (SEQ ID NO:8),

YTCAATAGGRCTAATKGGRAAATTTAAAGTRCA (SEQ ID NO:9),
YTCTGTCAATGGCCATTGYTTRACYYTTGGGCC (SEQ ID NO:10),
TKTACAWATYTCTRYTAATGCTTTTATTTTYTC (SEQ ID NO:11),
AAYTYTTGAAATYTTYCCTTCCTTTTCCATHTC (SEQ ID NO:12),
AAATAYKGGAGTATTRTATGGATTYTCAGGCCC (SEQ ID NO:13),
TCTCCAYTTRGTRCTGTCYTTTTTCTTTATRGC (SEQ ID NO:14),
TYTYYTATTAAGYTCYCTGAAATCTACTARTTT (SEQ ID NO:15),
TKTTYTAAARGGYTCYAAGATTTTTGTCATRCT (SEQ ID NO:16),
CATGTATTGATADATRAYYATKTCTGGATTTTG (SEQ ID NO:17),
TATYTCTAARTCAGAYCCTACATACAAATCATC (SEQ ID NO:18),
TCTYARYTCCTCTATTTTTGYTCTATGCTGYYC (SEQ ID NO:19),
AAGRAATGGRGGTTCTTTCTGATGYTTYTTRTC (SEQ ID NO:20),
TRGCTGCYCCATCTACATAGAAVGTTTCTGCWC (SEQ ID NO:21),
GACAACYTTYTGTCTTCCAYTGTYAGTWASATA (SEQ ID NO:22),
YGAATCCTGYAAVGCTARRTDAATTGCTTGTAA (SEQ ID NO:23),
YTGTGARTCTGTYACTATRTTTACTTCTRRTCC (SEQ ID NO:24),
TATTATTTGAYTRACWAWCTCTGATTCACTYTK (SEQ ID NO:25),
CAGRTARACYTTTTCCTTTTTTATTARYTGYTC (SEQ ID NO:26),
TCCTCCAATYCCTTTRTGTGCTGGTACCCATGM (SEQ ID NO:27),
TCCHBBACTGACTAATYTATCTACTTGTTCATT (SEQ ID NO:28),
ATCTATTCCATYYAAAAATAGYAYYTTYCTGAT (SEQ ID NO:29),
GTGGYAGRTTAAARTCAYTAGCCATTGCTYTCC (SEQ ID NO:30),
CACAGCTRGCTACTATTTCYTTYGCTACYAYRG (SEQ ID NO:31),
RYTGCCATATYCCKGGRCTACARTCTACTTGTC (SEQ ID NO:32),
DGATWAYTTTTCCTTCYARATGTGTACAATCTA (SEQ ID NO:33),
CTATRTAKCCACTRGCYACATGRACTGCTACYA (SEQ ID NO:34),
CYTGYCCTGTYTCTGCTGGRATDACTTCTGCTT (SEQ ID NO:35),
TGSKGCCATTGTCTGTATGTAYTRYTKTTACTG (SEQ ID NO:36),
GAATKCCAAATTCCTGYTTRATHCCHGCCCACC (SEQ ID NO:37),
ATTCYAYTACYCCTTGACTTTGGGGRTTGTAGG (SEQ ID NO:38),
GBCCTATRATTTKCTTTAATTCHTTATTCATAG (SEQ ID NO:39),
CTSTCTTAAGRTGYTCAGCYTGMTCTCTTACYT (SEQ ID NO:40),
TAAAATTGTGRATRAAYACTGCCATTTGTACWG (SEQ ID NO:41),
CTGCACTGTAYCCCCCAATCCCCCYTYTTCTTT (SEQ ID NO:42),
TGTCTGTWGCTATYATRYCTAYTATTCTYTCCC (SEQ ID NO:43),

TTRTRATTTGYTTTTGTARTTCTYTARTTTGTA (SEQ ID NO:44).

15. The set of synthetic oligonucleotides of claim 14, wherein said second segment comprises AGGCATAGGACCCGTGTCTT (SEQ ID NO:55).

16. A set of synthetic oligonucleotides useful as capture probes in a sandwich hybridization assay for HIV, comprising two oligonucleotides, wherein each member of the set comprises

a first segment comprising a nucleotide sequence substantially complementary to a segment of HIV nucleic acid; and

a second segment comprising a nucleotide sequence substantially complementary to an oligonucleotide bound to a solid phase,

wherein said HIV nucleic acid segments are
CATCTGCTCCTGTRTCTAATAGAGCTTCYTTTA (SEQ ID NO:45),
ATCCATYCCTGGCTTTAATTTTACTGGTACAGT (SEQ ID NO:46),
TATTCCTAAYTGRACTTCCCARAARTCYTGAGT (SEQ ID NO:47),
ACWYTGGAATATYGCYGGTGATCCTTTCCAYCC (SEQ ID NO:48),
CCATTTRTCAGGRTGGAGTTCATAMCCCATCCA (SEQ ID NO:49),
CTAYTATGGGKTCYKTYTCTAACTGGTACCAYA (SEQ ID NO:50),
ATCTGGTTGTGCTTGAATRATYCCYARTGCATA (SEQ ID NO:51),
CATGCATGGCTTCYCCTTTTAGYTGRCATTTAT (SEQ ID NO:52),
AACAGGCDGCYTTAACYGYAGYACTGGTGAAAT (SEQ ID NO:53),
TGTCYCTGTAATAAACCCGAAAATTTTGAATTT (SEQ ID NO:54).

17. The set of synthetic oligonucleotides of claim 16, wherein said second segment comprises

CTTCTTTGGAGAAAGTGGTG (SEQ ID NO:56).

18. A set of synthetic oligonucleotides useful as a spacer oligonucleotide in a sandwich hybridization

assay for HIV, comprising two oligonucleotides, wherein the synthetic oligonucieotide comprises a segment substantially complementary to a segment of HIV nucleic acid, wherein said HIV nucleic acid segments are TATAGCTTTHTDTCCRCAGATTTCTAYRR (SEQ ID NO:57), VCCAAKCTGRGTCAACADATTTCKTCCRATTAT (SEQ ID NO:58), TGGTGTGGTAARYCCCCACYTYAAYAGATGYYS (SEQ ID NO:59), TCCTGCTTTTCCYWDTYTAGTYTCYCTRY (SEQ ID NO:60), YTCAGTYTTCTGATTTGTYGTDTBHKTNADRGD (SEQ ID NO:61), AATTRYTGTGATATTTYTCATGDTCHTCTTGRGCCTT (SEQ ID NO:62), GCCATCTKCCTGCTAATTTTARDAKRAARTATGCTGTYT (SEQ ID NO:63).

19. A solution sandwich hybridization assay for detecting the presence of HIV in a sample, comprising

(a) contacting the sample under hybridizing conditions with an excess of (i) amplifier probe comprising the set of synthetic oligonucleotides of claim 10 and (ii) a set of capture probe oligonucleotides wherein the capture probe oligonucleotide comprises a first segment comprising a nucleotide sequence that is substantially complementary to a segment of HIV-1 nucleic acid and a second segment that is substantially complementary to an oligonucleotide bound to a solid phase;

(b) contacting the product of step (a) under hybridizing conditions with said oligonucleotide bound to the solid phase;

(c) thereafter separating materials not bound to the solid phase;

(d) contacting the bound product of step (c) under hybridization conditions with the nucleic acid multimer, said multimer comprising at least one oligonucleotide unit that is substantially complementary to the second segment of the amplifier probe polynucleotide and a multiplicity of second

oligonucleotide units that are substantially complementary to a labeled oligonucleotide;

(e) removing unbound multimer;

(f) contacting under hybridizing conditions the solid phase complex product of step (e) with the labeled oligonucleotide;

(g) removing unbound labeled oligonucleotide; and

(h) detecting the presence of label in the solid phase complex product of step (g).

20. A solution sandwich hybridization assay for detecting the presence of HIV in a sample, comprising

(a) contacting the sample under hybridizing conditions with an excess of (i) amplifier probe comprising the set of synthetic oligonucleotides of claim 14 and (ii) a set of capture probe oligonucleotides wherein the capture probe oligonucleotide comprises a first segment comprising a nucleotide sequence that is substantially complementary to a segment of HIV nucleic acid and a second segment that is substantially complementary to an oligonucleotide bound to a solid phase;

(b) contacting the product of step (a) under hybridizing conditions with said oligonucleotide bound to the solid phase;

(c) thereafter separating materials not bound to the solid phase;

(d) contacting the bound product of step (c) under hybridization conditions with the nucleic acid multimer, said multimer comprising at least one oligonucleotide unit that is substantially complementary to the second segment of the amplifier probe polynucleotide and a multiplicity of second

oligonucleotide units that are substantially complementary to a labeled oligonucleotide;

(e) removing unbound multimer;

(f) contacting under hybridizing conditions the solid phase complex product of step (e) with the labeled oligonucleotide;

(g) removing unbound labeled oligonucleotide; and

(h) detecting the presence of label in the solid phase complex product of step (g).

21. The solution sandwich hybridization assay of claim 19, wherein step (a) further comprises contacting said sample with a set of synthetic oligonucleotides useful as spacer oligonucleotides in a sandwich hybridization assay for HIV, said set comprising two oligonucleotides, wherein the synthetic oligonucleotide comprises a segment substantially complementary to a segment of HIV nucleic acid, wherein said HIV nucleic acid segments are TATAGCTTTHTDTCCRCAGATTTCTAYRR (SEQ ID NO:57), VCCAAKCTGRGTCAACADATTTCKTCCRATTAT (SEQ ID NO:58), TGGTGTGGTAARYCCCCACYTYAAYAGATGYYS (SEQ ID NO:59), TCCTGCTTTTCCYWDTYTAGTYTCYCTRY (SEQ ID NO:60), YTCAGTYTTCTGATTTGTYGTDTBHKTNADRGD (SEQ ID NO:61), AATTRYTGTGATATTTYTCATGDTCHTCTTGRGCCTT (SEQ ID NO:62), GCCATCTKCCTGCTAATTTTARDAKRAARTATGCTGTYT (SEQ ID NO:63).

22. The solution sandwich hybridization assay of claim 20, wherein step (a) further comprises contacting said sample with a set of synthetic oligonucleotides useful as spacer oligonucleotides in a sandwich hybridization assay for HIV, comprising two oligonucleotides, wherein the synthetic oligonucleotide comprises a segment substantially complementary to a

segment of HIV nucleic acid, wherein said HIV segments are
TATAGCTTTHTDTCCRCAGATTTCTAYRR (SEQ ID NO:57),
VCCAAKCTGRGTCAACADATTTCKTCCRATTAT (SEQ ID NO:58),
TGGTGTGGTAARYCCCCACYTYAAYAGATGYYS (SEQ ID NO:59),
TCCTGCTTTTCCYWDTYTAGTYTCYCTRY (SEQ ID NO:60),
YTCAGTYTTCTGATTTGTYGTDTBHKTNADRGD (SEQ ID NO:61),
AATTRYTGTGATATTTYTCATGDTCHTCTTGRGCCTT (SEQ ID NO:62),
GCCATCTKCCTGCTAATTTTARDAKRAARTATGCTGTYT (SEQ ID NO:63).

23. A solution sandwich hybridization assay for detecting the presence of HIV in a sample, comprising

(a) contacting the sample under hybridizing conditions with an excess of (i) a set of amplifier probe oligonucleotides wherein the amplifier probe oligonucleotide comprises a first segment comprising a nucleotide sequence substantially complementary to a segment of HIV nucleic acid and a second segment comprising a nucleotide sequence substantially complementary to an oligonucleotide unit of a nucleic acid multimer and (ii) capture probes comprising the set of synthetic oligonucleotides of claim 12;

(b) contacting the product of step (a) under hybridizing conditions with said oligonucleotide bound to the solid phase;

(c) thereafter separating materials not bound to the solid phase;

(d) contacting the bound product of step (c) under hybridization conditions with the nucleic acid multimer, said multimer comprising at least one oligonucleotide unit that is substantially complementary to the second segment of the amplifier probe polynucleotide and a multiplicity of second

oligonucleotide units that are substantially complementary to a labeled oligonucleotide;

(e) removing unbound multimer;

(f) contacting under hybridizing conditions the solid phase complex product of step (e) with the labeled oligonucleotide;

(g) removing unbound labeled oligonucleotide; and

(h) detecting the presence of label in the solid phase complex product of step (g).

24. A solution sandwich hybridization assay for detecting the presence of HIV in a sample, comprising

(a) contacting the sample under hybridizing conditions with an excess of (i) a set of amplifier probe oligonucleotides wherein the amplifier probe oligonucleotide comprises a first segment comprising a nucleotide sequence substantially complementary to a segment of HIV nucleic acid and a second segment comprising a nucleotide sequence substantially complementary to an oligonucleotide unit of a nucleic acid multimer and (ii) capture probes comprising the set of synthetic oligonucleotides of claim 16;

(b) contacting the product of step (a) under hybridizing conditions with said oligonucleotide bound to the solid phase;

(c) thereafter separating materials not bound to the solid phase;

(d) contacting the bound product of step (c) under hybridization conditions with the nucleic acid multimer, said multimer comprising at least one oligonucleotide unit that is substantially complementary to the second segment of the amplifier probe polynucleotide and a multiplicity of second

oligonucleotide units that are substantially complementary to a labeled oligonucleotide;

(e) removing unbound multimer;

(f) contacting under hybridizing conditions the solid phase complex product of step (e) with the labeled oligonucleotide;

(g) removing unbound labeled oligonucleotide; and

(h) detecting the presence of label in the solid phase complex product of step (g).

25. The solution sandwich hybridization assay of claim 23, wherein step (a) further comprises contacting said sample with the set of a set of synthetic oligonucleotides useful as a spacer oligonucleotide in a sandwich hybridization assay for HIV, comprising two oligonucleotides, wherein the synthetic oligonucleotide comprises a segment substantially complementary to a segment of HIV nucleic acid, wherein said HIV nucleic acid segments are
TATAGCTTTHTDTCCRCAGATTTCTAYRR (SEQ ID NO:57),
VCCAAKCTGRGTCAACADATTTCKTCCRATTAT (SEQ ID NO:58),
TGGTGTGGTAARYCCCCACYTYAAYAGATGYYS (SEQ ID NO:59),
TCCTGCTTTTCCYWDTYTAGTYTCYCTRY (SEQ ID NO:60),
YTCAGTYTTCTGATTTGTYGTDTBHKTNADRGD (SEQ ID NO:61),
AATTRYTGTGATATTTYTCATGDTCHTCTTGRGCCTT (SEQ ID NO:62),
GCCATCTKCCTGCTAATTTTARDAKRAARTATGCTGTYT (SEQ ID NO:63).

26. The solution sandwich hybridization assay of claim 24, wherein step (a) further comprises contacting said sample with the set of a set of synthetic oligonucleotides useful as a spacer oligonucleotide in a sandwich hybridization assay for HIV, comprising two oligonucleotides, wherein the synthetic oligonucleotide comprises a segment substantially complementary to a

segment of HIV nucleic acid, wherein said HIV nucleic acid segments are TATAGCTTTHTDTCCRCAGATTTCTAYRR (SEQ ID NO:57), VCCAAKCTGRGTCAACADATTTCKTCCRATTAT (SEQ ID NO:58), TGGTGTGGTAARYCCCCACYTYAAYAGATGYYS (SEQ ID NO:59), TCCTGCTTTTCCYWDTYTAGTYTCYCTRY (SEQ ID NO:60), YTCAGTYTTCTGATTTGTYGTDTBHKTNADRGD (SEQ ID NO:61), AATTRYTGTGATATTTYTCATGDTCHTCTTGRGCCTT (SEQ ID NO:62), GCCATCTKCCTGCTAATTTTARDAKRAARTATGCTGTYT (SEQ ID NO:63).

27. A kit for the detection of HIV in a sample comprising in combination

(i) a set of amplifier probe oligonucleotides wherein the amplifier probe oligonucleotide comprises a first segment comprising a nucleotide sequence substantially complementary to a segment of HIV nucleic acid and a second segment comprising a nucleotide sequence substantially complementary to an oligonucleotide unit of a nucleic acid multimer;

(ii) a set of capture probe oligonucleotides wherein the capture probe oligonucleotide comprises a first segment comprising a nucleotide sequence that is substantially complementary to a segment of HIV nucleic acid and a second segment that is substantially complementary to an oligonucleotide bound to a solid phase;

(iii) a nucleic acid multimer, said multimer comprising at least one oligonucleotide unit that is substantially complementary to the second segment of the amplifier probe polynucleotide and a multiplicity of second oligonucleotide units that are substantially complementary to a labeled oligonucleotide; and

(iv) a labeled oligonucleotide.

28. The kit of claim 27, further comprising a set of spacer oligonucleotides, wherein said spacer oligonucleotide is selected from the group comprising TATAGCTTTHTDTCCRCAGATTTCTAYRR (SEQ ID NO:57), VCCAAKCTGRGTCAACADATTTCKTCCRATTAT (SEQ ID NO:58), TGGTGTGGTAARYCCCCACYTYAAYAGATGYYS (SEQ ID NO:59), TCCTGCTTTTCCYWDTYTAGTYTCYCTRY (SEQ ID NO:60), YTCAGTYTTCTGATTTGTYGTDTBHKTNADRGD (SEQ ID NO:61), AATTRYTGTGATATTTYTCATGDTCHTCTTGRGCCTT (SEQ ID NO:62), GCCATCTKCCTGCTAATTTTARDAKRAARTATGCTGTYT (SEQ ID NO:63).

29. The kit of claim 27, wherein said set of amplifier probe oligonucleotides is the set of claim 10.

30. The kit of claim 27, wherein said set of amplifier probe oligonucleotides is the set of claim 14.

31. The kit of claim 27, wherein said set of capture probe oligonucleotides is the set of claim 12.

32. The kit of claim 27, wherein said set of capture probe oligonucleotides is the set of claim 16.

33. The kit of claim 27, further comprising instructions for the use thereof.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US92/11168

A. CLASSIFICATION OF SUBJECT MATTER

IPC(5) :C12Q 1/68; C07H 21/04
US CL :435/5, 6; 536/23.1, 23.72, 24.3

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 435/5, 6; 536/23.1, 23.72, 24.3

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

MEDLINE, APS, EMBASE, BIOSIS
search terms: HIV, sandwich or solution hybridization, capture probe

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO,A, 89/03891 (Urdea et al.) 05 May 1989, pages 23-31.	1-33
Y	Nature, Volume 313, issued 24 January 1985, Ratner, Lee et al, "Complete nucleotide sequence of the AIDS virus, HTLV-III", pages 277-283, especially figures 1 and 3.	1-33
Y	EP, A, 0318245 (Hogan et al.), 31 May 1989, page 5, lines 16-29.	9,18,21,22, 25,26,28-33
Y,P	US, A, 5,124,246 (Urdea et al) 23 June 1992, columns 2 and 3.	1-33
Y	US, A, 5,008,182 (Sninsky et al) 16 April 1991, columns 2, 4 and 5.	1-33

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be part of particular relevance

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"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
17 February 1993	ISA/US 05 MAR 1993
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